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DATE: Wednesday, November 09, 2005

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	<i>DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR</i>		
<input type="checkbox"/>	L1	(hsp\$ or cpn or chaperon\$ or (heat near shock) or (stress near protein) or (stress near peptide) or (stress near polypeptide) or (shock near protein)).ti,ab,clm.	7814
<input type="checkbox"/>	L2	(complex or noncovalent\$ or complexes or non-covalent\$ or associat\$ or linked or linker or fusion or fused or conjugate or conjugation or couple or coupled or attach\$ or joiner or joined or links or complexed).ti,ab,clm.	5056715
<input type="checkbox"/>	L3	L2 same l1	1486
<input type="checkbox"/>	L4	(vaccine or composition or immunogen or immunogenic or isolated or purified or extract or extracted or lysate or pharmaceutical\$).ti,ab,clm.	2560726
<input type="checkbox"/>	L5	L4 same l3	558
<input type="checkbox"/>	L6	L5 and (pylori or helicobacter or felis or hpylori or pylori or pylorum or pyloridis or pylon).clm.	5
<input type="checkbox"/>	L7	(hspa or hspb or hsp or hsp-a or hsp-b).clm. same (ure or urea or ureb or ure-a or urease or ureaseb or ureasea).clm. and (helicobacter or pylori)	3
<input type="checkbox"/>	L8	l1 same (noncovalent\$ or non-covalent\$ or complex\$).ti,ab,clm.	524
<input type="checkbox"/>	L9	l8 same vaccine.ti,ab,clm.	49
<input type="checkbox"/>	L10	(fimch or fim-ch or (fimh same fimc) or (fim-h same fim-c) or fim-hc or fimhc).clm.	5
<input type="checkbox"/>	L11	(fimc or fim-C).clm. and (fimh or fim-h).clm. and l2	5
<input type="checkbox"/>	L12	L11	5
<input type="checkbox"/>	L13	L12 not l10	0
<input type="checkbox"/>	L14	adhesin.clm. same chaperon\$.clm. nopt l10	92
<input type="checkbox"/>	L15	adhesin.clm. same chaperon\$.clm. not l10	1

END OF SEARCH HISTORY

et Items Description
S1 3067 E2-E23
S2 15326 E1-E50
S3 394244 'CHAPERONIN' OR DC='D4.680' OR R4:R7
S4 5 'CHAPERONIN COMPLEXES'
S5 4 'CHAPERONIN CPN60': 'CHAPERONIN CPN60 3'
S6 1 'CHAPERONIN GRO-EL OVERPRODUCTION' OR 'CHAPERONIN GRO-ES O-
 VERPRODUCTION'
S7 17 'CHAPERONIN PROTEIN': 'CHAPERONIN PROTEIN SUBUNITS'
S8 410424 S1 OR S2 OR S3 OR S5 OR S6 OR S7
S9 472405 S8 OR CHAPERON?
S10 4599 S9 (3N) COMPLEX?
S11 0 S10 AND SONICAT? AND CENTRIFUG?
S12 515 S10 (50N) (ISOLAT? OR PURIF? OR IMMUNI?)
S13 1 S10 AND SONICAT?

? s s12/1999:2005

Processing

Processed 10 of 26 files ...

>>>One or more prefixes are unsupported

>>> or undefined in one or more files.

>>>Year ranges not supported in one or more files

Completed processing all files

511 S12

35188059 PY=1999 : PY=2005

S14 317 S12/1999:2005

? s s12 not s14

515 S12

317 S14

S15 198 S12 NOT S14

? rd

...examined 50 records (50)

...examined 50 records (100)

...examined 50 records (150)

...completed examining records

S16 72 RD (unique items)

?

DIAGNOSIS
Search notes
1/5/05

16/9/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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12450820 PMID: 9764760

Purification and characterization of chaperonins 60 and 10 from *Methylobacillus glycogenes*.

Kawata Y; Doi K; Omoto H; Mizobata T; Nagai J
Department of Biotechnology, Faculty of Engineering, Tottori University,
Japan. kawata@bio.tottori-u.ac.jp

Cell stress & chaperones (UNITED STATES) Sep 1998, 3 (3) p200-7,
ISSN 1355-8145 Journal Code: 9610925

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Two proteins belonging to the group I chaperonin family were isolated from an obligate methanotroph, *Methylobacillus glycogenes*. The two proteins, one a GroEL homologue (cpn60: *M. glycogenes* 60 kDa chaperonin) and the other a GroES homologue (cpn10: *M. glycogenes* 10 kDa chaperonin), composed a heteropolymeric complex in the presence of ATP. Both proteins were purified from crude extracts of *M. glycogenes* by anion-exchange (DEAE-Toyopearl) and gel-filtration (Sephacryl S-400) chromatography. The native molecular weights of each chaperonin protein as determined by high-performance liquid chromatography (HPLC) gel-filtration were 820 000 for cpn60 and 65 000 for cpn10. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed that the subunit molecular weights of cpn60 and cpn10 were 58 000 and 10 000, respectively. Both cpn60 and cpn10 possessed amino acid sequences which were highly homologous to other group I chaperonins. *M. glycogenes* cpn60 displayed an ATPase activity which was inhibited in the presence of cpn10. The chaperonins also displayed an ability to interact with and facilitate the refolding of *Thermus malate dehydrogenase* and yeast enolase in a manner similar to that of GroEL/ES. The similarities between the *Escherichia coli* GroE proteins are discussed.

Tags: Comparative Study; Research Support, Non-U.S. Gov't

Descriptors: *Bacterial Proteins--isolation and purification--IP; *Chaperonin 10--isolation and purification--IP; *Chaperonin 60--isolation and purification--IP; *Gram-Negative Aerobic Bacteria--chemistry--CH; Adenosinetriphosphatase--metabolism--ME; Amino Acid Sequence; Bacterial Proteins--chemistry--CH; Chaperonin 10--chemistry--CH; Chaperonin 60 --chemistry--CH; Chromatography, Gel; Chromatography, High Pressure Liquid; Chromatography, Ion Exchange; *Escherichia coli*--chemistry--CH; Fungal Proteins--chemistry--CH; GroEL Protein--isolation and purification--IP; GroES Protein--isolation and purification--IP; Humans; Malate Dehydrogenase --chemistry--CH; Molecular Sequence Data; Molecular Weight; Phosphopyruvate Hydratase--chemistry--CH; Protein Folding; *Saccharomyces cerevisiae* --chemistry--CH; Sequence Alignment; Sequence Homology, Amino Acid; Species Specificity

CAS Registry No.: 0 (Bacterial Proteins); 0 (Chaperonin 10); 0 (Chaperonin 60); 0 (Fungal Proteins); 0 (GroEL Protein); 0 (GroES Protein)

Enzyme No.: EC 1.1.1.37 (Malate Dehydrogenase); EC 3.6.1.3 (Adenosinetriphosphatase); EC 4.2.1.11 (Phosphopyruvate Hydratase)

Record Date Created: 19981230

Record Date Completed: 19981230

16/9/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

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12405444 PMID: 9714842

Characterization and sequence comparison of temperature-regulated chaperonins from the hyperthermophilic archaeon *Archaeoglobus fulgidus*.

Emmerhoff O J; Klenk H P; Birkeland N K

Department of Microbiology, University of Bergen, Jahnebakken 5, N-5020, Bergen, Norway.

Gene (NETHERLANDS) Jul 30 1998, 215 (2) p431-8, ISSN 0378-1119

Journal Code: 7706761

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

We have cloned and sequenced the genes encoding two chaperonin subunits (Cpn-alpha and Cpn-beta), from *Archaeoglobus fulgidus*, a sulfate-reducing hyperthermophilic archaeon. The genes encode proteins of 545 amino acids with calculated Mr of 58 977 and 59 683. Both proteins have been identified in cytoplasmic fractions of *A. fulgidus* by Western analysis using antibodies raised against one of the subunits expressed in *Escherichia coli*, and by N-terminal amino acid sequencing of chaperonin complexes purified by immunoprecipitation. The chaperonin genes appear to be under heat shock regulation, as both proteins accumulate following temperature shift-up of growing *A. fulgidus* cells, implying a role of the chaperonin in thermoadaptation. Canonical Box A and Box B archaeal promoter sequences, as well as additional conserved putative signal sequences, are located upstream of the start codons. A phylogenetic analysis using all the available archaeal chaperonin sequences, suggests that the alpha and beta subunits are the results of late gene duplications that took place well after the establishment of the main archaeal evolutionary lines.

Tags: Comparative Study

Descriptors: **Archaeoglobus fulgidus*--genetics--GE; *Chaperonins--chemistry--CH; *Chaperonins--genetics--GE; *Gene Expression Regulation, Archaeal; *Phylogeny; Amino Acid Sequence; *Archaeoglobus fulgidus*--classification--CL; *Archaeoglobus fulgidus*--growth and development--GD; Base Sequence; Chaperonins--biosynthesis--BI; Cloning, Molecular; DNA Primers; Genes, Structural; Heat; Molecular Sequence Data; Polymerase Chain Reaction; Recombinant Proteins--biosynthesis--BI; Recombinant Proteins--chemistry--CH

CAS Registry No.: 0 (Chaperonins); 0 (DNA Primers); 0 (Recombinant Proteins)

Record Date Created: 19981001

Record Date Completed: 19981001

16/9/6 (Item 6 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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12242306 PMID: 9545231

Ramifications of kinetic partitioning on usher-mediated pilus biogenesis.

Saulino E T; Thanassi D G; Pinkner J S; Hultgren S J

Department of Molecular Microbiology and Microbial Pathogenesis, Washington University School of Medicine, 660 South Euclid Avenue, Campus Box 8230, St Louis, MO 63110, USA.

EMBO journal (ENGLAND) Apr 15 1998, 17 (8) p2177-85, ISSN 0261-4189
Journal Code: 8208664

Contract/Grant No.: R01AI29549; AI; NIAID; R01DK51406; DK; NIDDK

Publishing Model Print

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

The biogenesis of diverse adhesive structures in a variety of Gram-negative bacterial species is dependent on the chaperone/usher pathway. Very little is known about how the usher protein translocates protein subunits across the outer membrane or how assembly of these adhesive structures occurs. We have discovered several mechanisms by which the usher protein acts to regulate the ordered assembly of type 1 pili, specifically through critical interactions of the chaperone-adhesin complex with the usher. A study of association and dissociation events of chaperone-subunit complexes with the usher in real time using surface plasmon resonance revealed that the **chaperone-adhesin complex** has the tightest and fastest association with the usher. This suggests that kinetic partitioning of **chaperone-adhesin complexes** to the usher is a defining factor in tip localization of the adhesin in the pilus. Furthermore, we identified and **purified** a chaperone-adhesin-usher assembly intermediate that was formed in vivo. Trypsin digestion assays showed that the usher in

this complex was in an altered conformation, which was maintained during pilus assembly. The data support a model in which binding of the **chaperone-adhesin complex** to the usher stabilizes the usher in an assembly-competent conformation and allows initiation of pilus assembly.

Tags: Research Support, U.S. Gov't, P.H.S.

Descriptors: *Bacterial Outer Membrane Proteins--metabolism--ME; *Endopeptidases; *Escherichia coli--metabolism--ME; *Escherichia coli Proteins; *Fimbriae Proteins; *Fimbriae, Bacterial--metabolism--ME; *Periplasmic Proteins; Adhesins, Bacterial--genetics--GE; Adhesins, Bacterial--isolation and purification--IP; Adhesins, Bacterial--metabolism--ME; Adhesins, Escherichia coli--metabolism--ME; Bacterial Outer Membrane Proteins--genetics--GE; Bacterial Outer Membrane Proteins--isolation and purification--IP; Bacterial Proteins--genetics--GE; Bacterial Proteins--isolation and purification--IP; Bacterial Proteins--metabolism--ME; Kinetics; Molecular Chaperones--metabolism--ME; Porins--metabolism--ME; Recombinant Fusion Proteins--genetics--GE; Recombinant Fusion Proteins--isolation and purification--IP; Recombinant Fusion Proteins--metabolism--ME; Time Factors; Trypsin--metabolism--ME

CAS Registry No.: 0 (Adhesins, Bacterial); 0 (Adhesins, Escherichia coli); 0 (Bacterial Outer Membrane Proteins); 0 (Bacterial Proteins); 0 (Escherichia coli Proteins); 0 (Molecular Chaperones); 0 (PapD protein, E coli); 0 (PapG protein, E coli); 0 (Periplasmic Proteins); 0 (Porins); 0 (Recombinant Fusion Proteins); 0 (atpG protein, E coli); 0 (fimC protein, E coli); 0 (fimC protein, bacteria); 0 (fimD protein, E coli); 0 (fimD protein, bacteria); 0 (fimH protein, E coli); 0 (fimbrillin); 147680-16-8 (Fimbriae Proteins)

Enzyme No.: EC 3.4.- (Endopeptidases); EC 3.4.- (prepilin peptidase protein, Bacteria); EC 3.4.21.4 (Trypsin)

Record Date Created: 19980624

Record Date Completed: 19980624

16/9/10 (Item 10 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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11764006 PMID: 8985401

Formation of native hepatitis C virus glycoprotein complexes.

Deleersnyder V; Pillez A; Wychowski C; Blight K; Xu J; Hahn Y S; Rice C M; Dubuisson J

Unite d'oncologie moleculaire, CNRS-URA1160, Institut Pasteur de Lille, France.

Journal of virology (UNITED STATES) Jan 1997, 71 (1) p697-704,
ISSN 0022-538X Journal Code: 0113724

Contract/Grant No.: CA57973; CA; NCI

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

The hepatitis C virus (HCV) glycoproteins (E1 and E2) interact to form a heterodimeric complex, which has been proposed as a functional subunit of the HCV virion envelope. As examined in cell culture transient-expression assays, the formation of properly folded, noncovalently associated E1E2 complexes is a slow and inefficient process. Due to lack of appropriate immunological reagents, it has been difficult to distinguish between glycoprotein molecules that undergo productive folding and assembly from those which follow a nonproductive pathway leading to misfolding and aggregation. Here we report the isolation and characterization of a conformation-sensitive E2-reactive monoclonal antibody (H2). The H2 monoclonal antibody selectively recognizes slowly maturing E1E2 heterodimers which are noncovalently linked, protease resistant, and no longer associated with the endoplasmic reticulum chaperone calnexin. This complex probably represents the native prebudding form of the HCV glycoprotein heterodimer. Besides providing a novel reagent for basic studies on HCV virion assembly and entry, this monoclonal antibody should be useful for optimizing production and isolation of native HCV glycoprotein complexes for serodiagnostic and vaccine applications.

Tags: Research Support, U.S. Gov't, Non-P.H.S.; Research Support, U.S. Gov't, P.H.S.

Descriptors: *Hepacivirus--metabolism--ME; *Viral Envelope Proteins--metabolism--ME; Animals; Antibodies, Monoclonal--metabolism--ME; Cell Line; Cercopithecus aethiops; Hamsters; Hepacivirus--genetics--GE; Hepatitis C Antibodies--metabolism--ME; Humans; Precipitin Tests; RNA, Viral--metabolism--ME; Recombinant Fusion Proteins--genetics--GE; Recombinant Fusion Proteins--metabolism--ME; Subcellular Fractions; Tumor Cells, Cultured; Viral Envelope Proteins--genetics--GE; Virion

CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (E1 protein, hepatitis C virus); 0 (Hepatitis C Antibodies); 0 (RNA, Viral); 0 (Recombinant Fusion Proteins); 0 (Viral Envelope Proteins); 157184-61-7 (hepatitis C virus envelope 2 protein)

Record Date Created: 19970131

Record Date Completed: 19970131

16/9/11 (Item 11 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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11670014 PMID: 8986757

Chaperone activity and structure of monomeric polypeptide binding domains of GroEL.

Zahn R; Buckle A M; Perrett S; Johnson C M; Corrales F J; Golbik R; Fersht A R

Cambridge Centre for Protein Engineering, Department of Chemistry, University of Cambridge, United Kingdom.

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Dec 24 1996, 93 (26) p15024-9, ISSN 0027-8424
Journal Code: 7505876

Publishing Model Print; Comment in Proc Natl Acad Sci U S A. 1997 Jan

7;94(1) 7-8; Comment in PMID 8990150

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

The chaperonin GroEL is a large complex composed of 14 identical 57-kDa subunits that requires ATP and GroES for some of its activities. We find that a monomeric polypeptide corresponding to residues 191 to 345 has the activity of the tetradecamer both in facilitating the refolding of rhodanese and cyclophilin A in the absence of ATP and in catalyzing the unfolding of native barnase. Its crystal structure, solved at 2.5 Å resolution, shows a well-ordered domain with the same fold as in intact GroEL. We have thus isolated the active site of the complex allosteric molecular chaperone, which functions as a "minichaperone." This has mechanistic implications: the presence of a central cavity in the GroEL complex is not essential for those representative activities in vitro, and neither are the allosteric properties. The function of the allosteric behavior on the binding of GroES and ATP must be to regulate the affinity of the protein for its various substrates in vivo, where the cavity may also be required for special functions.

Tags: Research Support, Non-U.S. Gov't

Descriptors: *Amino Acid Isomerases--chemistry--CH; *Carrier Proteins--chemistry--CH; *GroEL Protein--chemistry--CH; *GroEL Protein--metabolism--ME; *Protein Folding; *Protein Structure, Secondary; Allosteric Regulation; Amino Acid Isomerases--metabolism--ME; Amino Acid Sequence; Base Sequence; Binding Sites; Carrier Proteins--metabolism--ME; Crystallography, X-Ray; DNA Primers; Escherichia coli--metabolism--ME; GroEL Protein--biosynthesis--BI; Kinetics; Models, Molecular; Molecular Sequence Data; Peptide Fragments--biosynthesis--BI; Peptide Fragments--chemistry--CH; Peptide Fragments--metabolism--ME; Peptidylprolyl Isomerase; Polymerase Chain Reaction; Protein Denaturation; Recombinant Proteins--biosynthesis--BI; Recombinant Proteins--chemistry--CH; Recombinant Proteins--metabolism--ME; Thiosulfate Sulfurtransferase--chemistry--CH; Thiosulfate Sulfurtransferase--metabolism--ME

CAS Registry No.: 0 (Carrier Proteins); 0 (DNA Primers); 0 (GroEL Protein); 0 (Peptide Fragments); 0 (Recombinant Proteins)

Enzyme No.: EC 2.8.1.1 (Thiosulfate Sulfurtransferase); EC 5.1.1.- (Amino Acid Isomerases); EC 5.2.1.8 (Peptidylprolyl Isomerase)

Record Date Created: 19970128

Record Date Completed: 19970128

16/9/15 (Item 15 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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11317049 PMID: 8636160

Mycobacterium tuberculosis 16-kDa antigen (Hsp16.3) functions as an oligomeric structure in vitro to suppress thermal aggregation.

Chang Z; Primm T P; Jakana J; Lee I H; Serysheva I; Chiu W; Gilbert H F; Quijcho F A

Howard Hughes Medical Institute, Verna and Marrs McLean Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77030, USA.

Journal of biological chemistry (UNITED STATES) Mar 22 1996, 271 (12)

p7218-23, ISSN 0021-9258 Journal Code: 2985121R

Contract/Grant No.: GM 40379; GM; NIGMS; RR02250; RR; NCRR

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Tuberculosis continues to be a major disease threatening millions of lives worldwide. Several antigens of *Mycobacterium tuberculosis*, identified by monoclonal antibodies, have been cloned and are being exploited in the development of improved vaccines and diagnostic reagents. We have expressed and purified the 16-kDa antigen, an immunodominant antigen with serodiagnostic value, which has been previously cloned and shown to share low sequence homology with the alpha-crystallin-related small heat shock protein family. Sedimentation equilibrium analytical ultracentrifugation and dynamic light scattering demonstrate the formation of a specific oligomer, 149 +/- 8 kDa, consisting of approximately nine monomers. In 4 M urea, a smaller oligomer of 47 +/- 6 kDa (or trimer) is produced. Analysis by electron cryomicroscopy reveals a triangular shaped oligomeric structure arising from the presence of three subparticles or globules. Taken together, the data suggest an antigen complex structure of a trimer of trimers. This antigen, independent of ATP addition, effectively suppresses the thermal aggregation of citrate synthase at 40 degrees C, indicating that it can function as a molecular chaperone in vitro. A complex between the antigen and heat-denatured citrate synthase can be detected and isolated using high performance liquid chromatography. We propose to rename the 16-kDa antigen Hsp16.3 to be consistent with other members of the small heat shock protein family.

Tags: Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, P.H.S.

Descriptors: *Bacterial Proteins--metabolism--ME; *Heat-Shock Proteins--metabolism--ME; *Mycobacterium tuberculosis--immunology--IM; Bacterial Proteins--chemistry--CH; Bacterial Proteins--isolation and purification--IP; Base Sequence; Biopolymers; Citrate (si)-Synthase--metabolism--ME; Cloning, Molecular; DNA Primers; Heat; Heat-Shock Proteins--chemistry--CH; Heat-Shock Proteins--genetics--GE; Heat-Shock Proteins--isolation and purification--IP; Microscopy, Electron; Molecular Sequence Data; Polymerase Chain Reaction

CAS Registry No.: 0 (Bacterial Proteins); 0 (Biopolymers); 0 (DNA Primers); 0 (Heat-Shock Proteins)

Enzyme No.: EC 4.1.3.7 (Citrate (si)-Synthase)

Record Date Created: 19960709

Record Date Completed: 19960709

16/9/23 (Item 23 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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10901919 PMID: 7892228

FimH adhesin of type 1 pili is assembled into a fibrillar tip structure in the Enterobacteriaceae.

Jones C H; Pinkner J S; Roth R; Heuser J; Nicholes A V; Abraham S N; Hultgren S J

Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110.

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Mar 14 1995, 92 (6) p2081-5, ISSN 0027-8424
Journal Code: 7505876

Contract/Grant No.: RO1AI29549; AI; NIAID; RO1GM29647; GM; NIGMS; +

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Type 1 pili are heteropolymeric mannose-binding fibers produced by all members of the Enterobacteriaceae family. The bulk of the fiber is composed of FimA. Two macromolecular complexes responsible for mediating an interaction with mannose-containing receptors were purified from fimA-Escherichia coli by mannose affinity chromatography and ion-exchange chromatography. One complex contained only the mannose-binding adhesin, FimH, associated with FimG, a minor component of the type 1 pilus. In the other complex the FimG-FimH moiety was loosely associated with a chaperone-minor subunit complex (FimC-FimF), possibly representing an intermediate in tip fibrilla assembly. The FimC chaperone has also been shown to form a preassembly complex with FimH that has been purified and characterized previously. Purified FimC did not bind to the FimG-FimH complex but did recognize FimH dissociated from the FimG-FimH complex. Quick-freeze deep-etch electron microscopy revealed that the FimG-FimH complex had a thin fibrillar architecture. High-resolution electron microscopy of type 1 pili revealed that a 16-nm fibrillar tip structure with an architecture identical to that of the FimG-FimH complex was joined end-to-end to the pilus rod. In a fimH- deletion mutant, the tip fibrillae joined to pilus rods were approximately 3 nm in length. The full-length tip fibrilla was restored by complementation with the fimH gene in trans. The bipartite nature of the type 1 pilus was also demonstrated on pili purified from clinical isolates of members of the Enterobacteriaceae family arguing that it is a conserved feature of the type 1 pilus.

Tags: Research Support, U.S. Gov't, P.H.S.

Descriptors: *Adhesins, Escherichia coli; *Bacterial Adhesion; *Bacterial Proteins--biosynthesis--BI; *Enterobacteriaceae--metabolism--ME; *Fimbriae Proteins; *Fimbriae, Bacterial--metabolism--ME; Bacterial Outer Membrane Proteins--isolation and purification--IP; Bacterial Outer Membrane Proteins--metabolism--ME; Bacterial Proteins--isolation and purification--IP; Chromatography, Affinity; Citrobacter freundii--genetics--GE; Citrobacter freundii--isolation and purification--IP; Citrobacter freundii--metabolism--ME; Enterobacter cloacae--genetics--GE; Enterobacter cloacae--isolation and purification--IP; Enterobacter cloacae--metabolism--ME; Enterobacteriaceae--genetics--GE; Escherichia coli--genetics--GE; Fimbriae, Bacterial--ultrastructure--UL; Freeze Etching; Humans; Klebsiella pneumoniae--genetics--GE; Klebsiella pneumoniae --isolation and purification--IP; Klebsiella pneumoniae--metabolism--ME; Mannose; Microscopy, Electron; Recombinant Proteins--biosynthesis--BI; Recombinant Proteins--isolation and purification--IP; Recombinant Proteins--metabolism--ME; Urinary Tract Infections--microbiology--MI

CAS Registry No.: 0 (Adhesins, Escherichia coli); 0 (Bacterial Outer Membrane Proteins); 0 (Bacterial Proteins); 0 (Recombinant Proteins); 0 (fimC protein, E coli); 0 (fimC protein, bacteria); 0 (fimH protein, E coli); 147680-16-8 (Fimbriae Proteins); 31103-86-3 (Mannose)

Record Date Created: 19950420

Record Date Completed: 19950420

16/9/53 (Item 8 from file: 35)

DIALOG(R)File 35:Dissertation Abs Online

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01134925 ORDER NO: AAD90-33939

MOLECULAR CHAPERONES AND THE EARLY STEPS OF PROTEIN TRANSLOCATION IN ESCHERICHIA COLI

Author: LECKER, STEWART HARRIS

Degree: PH.D.

Year: 1990

Corporate Source/Institution: UNIVERSITY OF CALIFORNIA, LOS ANGELES (0031)

Chair: WILLIAM WICKNER

Source: VOLUME 51/07-B OF DISSERTATION ABSTRACTS INTERNATIONAL.
PAGE 3253. 202 PAGES
Descriptors: BIOLOGY, MOLECULAR; CHEMISTRY, BIOCHEMISTRY
Descriptor Codes: 0307; 0487

The mechanism by which proteins are targeted and assembled into their correct cellular compartments is a fundamental process of cell biology. Molecular chaperones are proteins which transiently act to stabilize other polypeptides during their folding and assembly. This work describes biochemical studies undertaken to elucidate the role of molecular chaperones in the early steps of bacterial protein secretion.

The molecular chaperones from E. coli--SecB, trigger factor and CroEL--were compared in their ability to form specific complexes with precursor proteins. Soluble, cytoplasmic proteins did not form stable complexes suggesting an early sorting mechanism in the secretion process. Precursor proteins isolated as complexes with chaperones are maintained in a translocation competent state. Biophysical studies delineated here have shown that for the precursor proOmpA, this competent state contains significant secondary and tertiary structure. It is likely that the chaperones maintain precursors such as proOmpA in a monomeric, nonaggregated state by shielding apolar domains on their surface.

Experiments integrating the early chaperone-preprotein complexes with later stages of the translocation process were performed. Both the precursor protein and the chaperone SecB have a direct affinity for the peripheral membrane component of the translocation machinery, SecA. SecA acts as both a high affinity receptor for the precursor complex, as well as a molecular chaperone capable of stabilizing the precursor protein. The hydrolysis of ATP by the SecA protein is believed to drive both the cascade of chaperone function and precursor release into the membrane for translocation.

The studies described in this thesis have helped to clarify the role of molecular chaperones in protein secretion. We have found evidence for chaperone functions at multiple steps: as a possible initial sorting mechanism between cytosolic and secreted proteins; as a direct stabilizer of preproteins prior to translocation and finally as the membrane receptor for the precursor protein.

16/9/54 (Item 1 from file: 65)
DIALOG(R) File 65:Inside Conferences
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00408481 INSIDE CONFERENCE ITEM ID: CN003899925
In vitro assembly, isolation, and characterization of nascent polypeptide-chain-binding complexes containing molecular chaperones
Hansen, W. J.; Lingappa, V. R.; Welch, W. J.
CONFERENCE: Biology of heat shock proteins and molecular chaperones-Meeting
ABSTRACTS OF PAPERS PRESENTED AT THE MEETING ON BIOLOGY OF HEAT SHOCK PROTEINS AND MOLECULAR CHAPERONES, 1994 P: 105
The Laboratory, 1994
LANGUAGE: English DOCUMENT TYPE: Conference Abstracts
CONFERENCE SPONSOR: Cold Spring Harbor Laboratory
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CONFERENCE DATE: May 1994 (199405) (199405)

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Cooperation of the molecular chaperone Ydj1 with specific Hsp70 homologs to suppress protein aggregation

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Ydj1p, a cytosolic DnaJ homolog from *Saccharomyces cerevisiae*, is demonstrated to function as a molecular chaperone. Purified Ydj1p formed complexes with non-native polypeptides and suppressed protein aggregation. Ydj1p cooperated with Ssa Hsp70 proteins in the prevention of protein aggregation, but not with the Ssb Hsp70 proteins. Cooperation between these different molecular chaperones was only observed in the presence of hydrolyzable ATP and correlated with the ability of Ydj1p to stimulate the ATPase activity of the Hsp70 homolog with which it was paired. The regulatory and chaperone activities of a eukaryotic DnaJ homolog thus act together to assist Hsp70 in modulating the conformation of proteins.

DESCRIPTORS:

DnaJ; Hsp70; Molecular chaperone; Protein folding

CLASSIFICATION CODE AND DESCRIPTION:

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DIALOG(R)File 135:NewsRx Weekly Reports

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0000016686 (THIS IS THE FULLTEXT)

Heat Shock Proteins Demonstrate Potential as Patient-Specific Treatments
Cancer Weekly, October 20, 1997, p.11

DOCUMENT TYPE: Editor's Choice LANGUAGE: English

RECORD TYPE: FULLTEXT

AUDIENCE: Professional

WORD COUNT: 617

TEXT: A powerful new approach to using immunotherapy for the treatment of a variety of cancers, including metastatic disease, has been developed at the University of Connecticut School of Medicine.

Researchers at the University's Center for Immunotherapy of Cancer and Infectious Diseases announced their technique, in which the individual's own immune cells are stimulated to eliminate cancerous cells from the body, in the journal *Science* (1997;278(5335)).

The paper, entitled "Immunotherapy of Tumors with Autologous Cancer-Derived Heat Shock Protein Preparations," described the use of protein complexes purified from tumor cells in the treatment of cancer and metastasis. Heat shock proteins (HSPs) are a family of molecular chaperones

present in all cells. In this study, 80 percent of HSP-treated mice survived for longer than 250 days compared to fewer than 20 percent of control mice.

The approach to cancer immunotherapy described in the report is medically significant because it is applicable to virtually all forms of cancer. Heat shock proteins can be purified from a tumor specimen removed by surgery or biopsy. The paper described the treatment of five different kinds of cancers in mice, including metastatic lung cancer, melanoma, and colon carcinoma.

The versatility of heat shock proteins in the treatment of cancer derives from their normal physiological role in cells. HSPs play a role in protein trafficking, whereby they keep other proteins in their correct shape and location. When these molecular chaperones are purified from cells, they bring along with them small fragments, or peptides, derived from other proteins expressed in that cell, providing a molecular "fingerprint" of the cell's content. Vaccination with the HSP-peptide complexes purified from cancer cells has the potential to specifically stimulate the immune system to attack cells bearing those peptides, i.e., the cancer cells themselves.

"The HSP-peptide complex vaccination is perhaps the first general principle of immunization which now has shown to be effective against a wide variety of pre-existing cancers," said Pramod K. Srivastava, Ph.D., of the University of Connecticut School of Medicine and principle investigator for the project. "Decades of research suggest that tumors are distinct from one individual to another, with each tumor containing its own unique set of mutations. The task of identifying each of these mutations in individual cancer patients - with the intent of targeting them immunotherapeutically - is therefore impractical. In contrast, the process of purifying HSPs is the same for each patient's tumor and is relatively simple."

This immunotherapeutic approach can target the cancer-specific antigens of any cancer cell without the need to identify what those antigens are. Moreover, the results of this study demonstrate that the vaccine is effective against the primary tumor and its metastases. In human clinical settings, metastatic cancer frequently is untreatable and fatal, even if the primary cancer has been removed successfully.

Antigenics, L.L.C., holds the commercial rights to the HSP-based vaccines against cancer and infectious diseases.

"We have initiated a Phase I trial in pancreatic cancer where HSP-based vaccines are being prepared from individual cancer patients' tumors. Phase Ib clinical trials for the treatment of melanoma and renal cell carcinoma are expected to start in the fourth quarter 1997," said Garo Armen, Ph.D, Antigenics.

"We anticipate that vaccinating cancer patients with purified chaperone-peptide complexes will lead to a decrease in the size of the tumor and will treat metastasis. This approach, alone or in combination with surgery and chemotherapeutic treatments should extend and improve the quality of life for patients with a wide variety of cancers."

Further, Armen said, "heat shock protein technology provides Antigenics with a platform that also has applications for the treatment of many infectious diseases."

DESCRIPTORS: news

SUBJECT HEADING: Cancer Therapies (HSP)

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16/9/60 (Item 1 from file: 144)

DIALOG(R) File 144:Pascal

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In vitro complex-formation between the molecular chaperone DnaK and staphylococcal Protein A derivatives produced in Escherichia coli and its use in the purification of DnaK

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Complex-formation between a truncated staphylococcal Protein A produced in Escherichia coli and a native E. coli molecular chaperone, DnaK, can be used for the purification of DnaK by IgG-affinity chromatography. The half-time constant for in vitro formation of the Protein A-DnaK complex is about 14 min. Complex-formation in the presence of ATP is faster, but pre-incubation of DnaK with ATP decreases the final amount of the complex. A second complex with a slower migration on native PAGE is formed when the ratio of DnaK to Protein A is increased. A derivative of Protein A, ZZ, which essentially contains only two modified domains of Protein A, did not bind DnaK. After insertion of a tryptophan-rich peptide close to the C-terminus, the resulting protein, ZZT SUB 3, became able to bind DnaK. The binding of these three proteins to DnaK correlates with proteolysis in E. coli, indicating a possible role for the binding of DnaK in the control of proteolysis.

English Descriptors: Heat shock protein; Chaperone ; Escherichia coli; Molecular complex ; Protein A; Recombinant protein; In vitro; Affinity chromatography; Binding capacity; Method; Purification

Broad Descriptors: Enterobacteriaceae; Bacteria; Enterobacteriaceae; Bacterie; Enterobacteriaceae; Bacteria

French Descriptors: Proteine choc thermique; Chaperon ; Escherichia coli; Complexe moleculaire; Proteine A; Proteine recombinante; In vitro; Chromatographie affinite; Capacite fixation; Methode; Purification ; Proteine DnaK

Classification Codes: 002A31C09C; 215

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DIALOG(R) File 149:TGG Health&Wellness DB(SM)

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Anti-Ro/SS-A positivity and heat shock protein antibodies in patients with normal-pressure glaucoma.

Wax, Martin B.; Tezel, Gulgun; Saito, Isao; Gupta, Radhey S.; Harley, John B.; Li, Zhengzhi; Romano, Carmelo

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TEXT:

* PURPOSE: To describe the clinical and laboratory findings in 10 patients with normal-pressure glaucoma and anti-Ro/SS-A positivity by enzyme-linked immunosorbent assay (ELISA) and to determine whether that positivity may be related to an autoimmune mechanism for the optic neuropathy.

* METHODS: In this prospective study, we evaluated ocular and systemic clinical findings of 10 patients with normal-pressure glaucoma and anti-Ro/SS-A positivity by ELISA, including sicca complex features. Ouchterlony immunodiffusion was performed to confirm the presence of antibodies for Ro/SS-A, and the presence of other serum antibodies and their possible cross-reactivities with Ro/SS-A were investigated.

* RESULTS: None of the 10 patients with normal-pressure glaucoma and anti-Ro/SS-A positivity (by ELISA) had clinical or laboratory signs of Sjogren syndrome or other connective tissue diseases. Only one of 10 patients had evidence of anti-Ro/SS-A antibodies by Ouchterlony immunodiffusion. All patients demonstrated serum immunoreactivity to bacterial heat shock protein 60 (hsp60) by Western blotting. Cross-reactivity between bacterial hsp60 and Ro/SS-A was demonstrated by Western blotting. Immunoreactivity to bacterial hsp60 by ELISA was significantly elevated in the sera of patients with normal-pressure glaucoma. Furthermore, patients with either normal-pressure or primary open-angle glaucoma had increased serum immunoreactivity to human hsp60.

* CONCLUSIONS: Anti-Ro/SS-A positivity by ELISA in 10 patients with normal-pressure glaucoma was associated with a high level of serum immunoreactivity to bacterial hsp60, which may indicate that their glaucomatous optic neuropathy involves an as yet unidentified autoimmune mechanism. The identification of autoantibodies that react with human hsp60 in patients with normal, pressure and primary open-angle glaucoma may signify a common finding associated with the glaucomatous optic neuropathy process in some patients and appears to be unrelated to intraocular pressure levels.

The progressive optic neuropathy that is accompanied by normal intraocular pressure, open iridocorneal angles, and no evidence of other systemic disease is commonly termed normal-pressure glaucoma. Its etiology is presently unknown. There is some evidence that a subset of these patients may have an autoimmune component to their disease. Cartwright and associates(1) have reported an association of normal-pressure glaucoma and immune-related disease by identifying a 30% prevalence of autoimmune disorders by epidemiologic criteria in patients with normal-pressure glaucoma. In addition, we have reported(2,3) an increased prevalence of nonorgan-specific autoantibodies, monoclonal paraproteinemia, and anti-rhodopsin antibodies in patients

with normal-pressure glaucoma. These findings may reflect an abnormality of the immune system in which aberrant autoimmunity is directed toward antigens of retinal or optic nerve, or both, in some patients with normal-pressure glaucoma. Among the non-organ-specific autoantibodies, antibodies to the extractable nuclear antigens, including Sjogren syndrome A and B antigens (SS-A and SS-13; also commonly called Ro and La antigens), have been found in 30% of patients with normal-pressure glaucoma.(2)

We have previously speculated(2) that non-organ-specific autoantibody production in patients with normal-pressure glaucoma may be a marker for the presence of other unidentified autoantibodies (for example, retinal, glial, or vascular autoantigens) and thus may provide clues to the presence of autoimmune-related optic neuropathy in these patients. We remain suspicious that optic neuropathy may be an organ-specific manifestation of an autoimmune-mediated process in some glaucomatous patients, particularly those with normal intraocular pressure.

In this report, we describe the clinical and laboratory findings of 10 patients with normal-pressure glaucoma who were positive for anti-Ro/SS-A by hospital-based qualitative solid-phase enzyme-linked immunosorbent assay (ELISA). None of these patients, who were anti-Ro/SS-A positive (by ELISA), had signs or symptoms of Sjogren syndrome or sicca complex. Furthermore, the inability to demonstrate the presence of anti-Ro/SS-A by precipitin reactivity in nine of 10 patients suggests that our hospital-based ELISA for anti-Ro/SS-A likely represented a false-positive result or low levels of this autoantibody in these patients. We sought to examine whether, in our patients, the presence of anti-Ro/SS-A binding by ELISA could be explained by the presence of another, as yet unidentified antibody in their sera that could cross-react with anti-Ro/SS-A. We further reasoned that such a putative autoantigen might be neural in origin and therefore initiated a series of experiments in which we examined the cross-reactivities of several autoantigens found in neural tissues with Ro/SS-A.

PATIENTS and METHODS

We describe 10 patients with clinical findings of normal-pressure glaucoma who were positive for anti-Ro/SS-A. The inclusion criteria for normal-pressure glaucoma consisted of the following: the presence of open iridocorneal angles; no evidence of intraocular pressure greater than 23 mm Hg; glaucomatous changes in both visual fields and optic nerve cupping; and the absence of alternative causes of optic neuropathy. Alternative causes of optic neuropathy such as meningeal disease, infection, inflammation, ischemia, demyelination, or compressive lesions were excluded by neuro-ophthalmologic examination with magnetic resonance imaging. Visual field loss of patients was evaluated with the Humphrey Field Analyzer 30-2 program or Goldmann manual perimetry. Kinetic visual field defects included reproducible nasal steps of at least 10 degrees in width or paracentral scotomas more than 5 degrees in width. Our criteria for visual

field abnormalities on the computerized perimetric test included a corrected pattern standard deviation with a P value of less than .05 or a Glaucoma Hemifield Test outside normal limits obtained with at least two reliable and reproducible visual field examinations. Visual field damage was considered to be progressed when there was a change in 5 or more points, with at least 3 being contiguous, compared with the patient's baseline values, based on the glaucoma change probability analysis (STATPAC). Optic disk evaluation was performed in 16 eyes of eight patients (mean age (+ or -) SD, 65.0 (+ or -) 16.1 years) with normal-pressure glaucoma and anti-Ro/SS-A positivity and in 119 eyes of 60 patients of similar age (mean age, 66.3 (+ or -) 13.6 years) with normal-pressure glaucoma without anti-Ro/SS-A positivity (Mann-Whitney U test, P = .63). Evaluation of the optic disks was assessed both by stereoscopic optic disk photography and subsequent morphometric analysis of these images as previously described.(4) Stereoscopic color optic disk photographs were used to assess the presence of nerve fiber layer hemorrhage and vascular narrowing. Localized arteriolar narrowing was considered to be present when the red blood column was narrower in the peripapillary retina than farther distally; generalized narrowing was considered when the arterial caliber seemed narrow throughout its length, as previously described by Rader and associates.(5) Morphometric. optic disk analysis was performed by one of us (G.T.) masked to diagnosis and laboratory results.

All patients were evaluated ophthalmologically and rheumatologically for the presence of sicca complex and other connective tissue diseases. The presence of xerophthalmia and xerostomia and of positive Schirmer test, rose bengal test, and minor salivary gland examination were considered as diagnostic features of sicca complex. The Schirmer test I was considered abnormal when there was less than 10 mm of wetting in 5 minutes. Histopathologic examination obtained by biopsy from the minor salivary glands of the lower lip was performed in four patients with xerostomia. The biopsy results were classified according to the criteria described by Tarpley and associates.(6)

Blood for laboratory tests was drawn after informed consent was obtained from all patients. Complete blood cell count, erythrocyte sedimentation rate by the Westergren technique, rheumatoid factor by the latex-fixation technique, serum complement, immune complex ((C.sub.1q)), anticardiolipin antibody, lupus anticoagulant factor, and cryoglobulin measurements were performed. The qualitative and quantitative presence of antinuclear antibodies was assessed by indirect immunofluorescence using HEP-2 substrate. Antibodies to the extractable nuclear antigens, Ro/SS-A, La/SS-B, U1 ribonuclear protein, and Smith antigen were assessed by ELISA kits in the Barnes Hospital laboratory. Results were reported as negative or weakly, moderately, or strongly positive using the control values provided by the manufacturers for their respective

ELISA kits. These serum samples were also analyzed by Ouchterlony immunodiffusion for anti-Ro/SS-A positivity. Testing for monoclonal proteins was performed using immunofixation method in 1% agarose gels whereby an antigen is fixed in the gel by an antibody through the formation of an insoluble immune complex. (7) Antibodies with specificity for antibody classes (IgG, IgA, IgM, IgD, and IgE) and types ((Kappa) and (Lambda)) were used.

For immunoblotting, bovine retinas were dissected from eyes obtained at a local abattoir within 3 hours of death and homogenized in ice-cold lysis buffer containing 2 mM (N, (2-hydroxyethyl)piperazine-N- (2-ethanesulfonic acid)), 2 mM ethylenediaminetetraacetic acid, pH 7.4, and protease inhibitors (50 (Micro)M of phenylmethylsulfonyl fluoride and 1 (Micro)g/ml each of aprotinin, antipain, bacitracin, bestatin, chymostatin, leupeptin, and pepstatin A) for 5 minutes at 4 C. After centrifugation at 1,000 g for 10 minutes, the pellet (consisting of nuclei and unbroken cells) was discarded, and the membrane fraction was pelleted by centrifugation at 35,000 g for 20 minutes. The supernatant or soluble fraction was saved and the membrane pellet washed twice with

Tris (hydroxymethyl) aminomethane-buffered saline solution (50 mM Tris and 154 mM sodium chloride, pH 7-4) and homogenized in lysis buffer. Fractions were stored at -80 C until use. The protein concentrations in the membrane and soluble fractions were determined using the bicinchoninic acid method.

Samples including bovine retinal proteins, two antigenically distinct species of purified human recombinant Ro/SS-A antigen with 52 kd and 60 kd molecular weights, (Biodesign Int, Kennebunk, Maine), human 60 kd heat shock protein (hsp60), (Stress Gen Biotechnologies Corp, Victoria, British Columbia, Canada) and bacterial hsp60 (GroEL; Boehringer Mannheim Corp, Indianapolis, Indiana) were separated by electrophoresis in 12% sodium dodecyl sulfate polyacrylamide gels at 160 V for 1 hour and electrophoretically transferred to polyvinylidene fluoride membranes at 40 V for 2 hours. After transfer, membranes were incubated in TTBS (50 mM Tris hydrochloride, 154 mM sodium chloride, and 0.1% Tween 20, pH 7-5) containing 5% nonfat dry milk for 20 minutes, then overnight in the same buffer containing a dilution of primary antibody and sodium azide. Primary antibodies were patient sera or polyclonal antibody against human calreticulin, polyclonal antibody against both bacterial and human hsp60, (SPA-804; Stress Gen Biotechnologies Corp, Victoria, British Columbia, Canada), monoclonal antibodies against bacterial hsp60 (anti-GroEL; Stress Gen Biotechnologies Corp, Victoria, British Columbia, Canada, and MabII-13, provided by R.S.G. (8)), and monoclonal antibody against glutamic acid decarboxylase (Fab-6). After several washes in TTBS, the membranes were incubated in TTBS and 5% dry milk containing goat anti-human IgG conjugated with horseradish peroxidase (1:2,000) for

2 hours. Immunoreactive bands were visualized by enhanced chemiluminescence using commercial reagents.

Immunoprecipitation and preadsorption of hsp60 from retinal preparation was accomplished as follows. For depletion of hsp60 from bovine retinal supernatant, the samples were incubated with sepharose beads containing a covalently attached monoclonal antibody to human hsp60 (MabII-13). The preadsorption was carried out at 4 C for 3 hours on a rotator. After this period, the beads were removed by centrifugation, and the supernatant was treated one more time with the fresh antibody-bound beads to remove any residual hsp60. This procedure was effective in removing more than 95% of bovine hsp60 from the retinal samples. (9,10)

Enzyme-linked immunosorbent assay for bacterial hsp60 was performed in the following manner. Bacterial hsp60 antigen, GroEL (50 ng) in carbonate buffer (50 mM sodium carbonate, pH 8.8) was added (1 (Micro)g/ml) to each well of 96-well plates and incubated overnight at 4 C. Each well was then filled with blocking buffer (50 mM sodium phosphate and 0.5 M sodium chloride, pH 7.2) containing 3% normal goat serum and 0.1% sodium azide and incubated at 37 C for at least 6 hours. Control plates prepared without hsp were treated identically. Patient sera diluted 1:5,000 in phosphate-buffered saline solution (50 mM sodium phosphate and 150 mM sodium chloride, pH 7.4) plus 1% normal goat serum and sodium azide were added to duplicate wells of hsp-coated plates and control plates and incubated overnight at 4 C. The patient serum was removed by washing once with distilled water and twice with phosphate-buffered saline solution, then secondary antibody (goat antihuman IgG conjugated with horseradish peroxidase) was added (1:2,000). After a 1-hour incubation at room temperature, the secondary antibody was washed three times with phosphate-buffered saline solution. Incubation with 100 (Micro)l of substrate buffer, including 6 (Micro)l of hydrogen peroxide and ABTS (2.2-azino-di-(1,3-ethyl-benzthiazolin-sulfonate(6)) diammonium salt), was performed for 40 minutes. The plate was read at 410 nM.

RESULTS

The following describes the clinical characteristics of 10 patients with normal-pressure glaucoma who were positive for anti-Ro/SS-A antibodies by hospital-based ELISA. All had excellent central visual acuity (range, 20/20 to 20/40), except one patient, whose poor visual acuity (20/200) was related to age-related macular degeneration. Three of the patients had a family history of glaucoma. History of migraine or cold intolerance was absent in all 10 patients. Two of the patients had a history of immune-related systemic diseases (Table 1), and two patients had the findings of intracranial small vessel disease on magnetic resonance imaging.

TABLE 1. Patient Characteristics

Patient No.,

Age (yrs), Race, Sex	Family History of Glaucoma	Systemic Disease
1, 57, M, M	-	--
2, 65, W, F	-	--
3, 82, W, F	-	Arthritis, chest carcinoma
4, 65, B, M	-	--
5, 89, W, M	-	--
6, 71, W, F	+	Mitral valve prolapse
7, 40, B, F	+	--
8, 67, W, M	-	Coronary artery disease, dementia
9, 51, W, M	-	Insulin-dependent diabetes mellitus, skin carcinoma
10, 71, W, M	+	--

Visual field defects of the patients were characteristic of normal-pressure glaucoma, (11) namely, their scotomas were generally deep, steep, and close to fixation. Optic disk findings of these patients with anti-Ro/SS-A positivity were compared with those of other patients with normal-pressure glaucoma and without anti-Ro/SS-A positivity. Nerve fiber layer hemorrhages were a more frequent finding in patients with normal-pressure glaucoma and anti-Ro/SS-A positivity than in other patients without anti-Ro/SS-A positivity (six eyes, 38% vs 19 eyes, 16%; chi-square test, $P = .037$). Localized retinal arteriolar narrowing was not different in two subgroups of eyes with normal-pressure glaucoma (11 eyes, 69% vs 69 eyes, 58%; chi-square test, $P = .41$); however, generalized retinal arteriolar narrowing was more frequently noted in anti-Ro/SS-A-positive patients (14 eyes, 88% vs 73 eyes, 61%; chi-square test, $P = .040$) (Figure 1). The mean ratio of neural rim area to disk area ($0.22 (+ \text{ or } -) 0.1$ vs $0.26 (+ \text{ or } -) 0.1$) and ratio of peripapillary chorioretinal atrophy area to disk area ($0.76 (+ \text{ or } -) 0.2$ vs $0.67 (+ \text{ or } -) 0.2$) were not different between two subgroups of eyes with normal-pressure glaucoma (Mann-Whitney U test, $P = .3$ and $P = .14$, respectively).

(Figure 1 ILLUSTRATION OMITTED)

Five of the patients had a positive clinical history of foreign-body sensation in the eye related to dry eye, and one of the patients had complaints of dry mouth. These positive histories, however, were not spontaneous complaints but rather were obtained after leading questions. The Schirmer test I was positive in four of these patients. Four patients with anti-Ro/SS-A positivity underwent lip biopsy, and the results of these procedures were negative (Table 2). These negative biopsy results prompted our cessation of this ancillary test. Rheumatologic and systemic evaluation of these patients disclosed that all four were asymptomatic for Sjogren syndrome, systemic lupus erythematosus, or the other connective tissue diseases, although one of the patients had some history of arthritis.

TABLE 2. Features of the Sicca Complex in Patients With Normal-pressure Glaucoma and Anti-Ro/SS-A Positivity
Lacrimal Gland Findings

Patient No.	Xerophthalmia	Schirmer Test	Rose Bengal Test
1	+	+	-
2	-	+	-

3	-	-	-
4	-	+	-
5	+	-	-
6	-	-	-
7	-	-	-
8	+	+	+
9	+	-	-
10	+	-	-

Salivary Gland Findings
Recurrent

Patient No.	Xerostomia	Enlargement	Biopsy
1	-	-	-
2	-	-	N/A
3	-	-	N/A
4	+	-	-
5	-	-	N/A
6	-	-	-
7	-	-	N/A
8	-	-	-
9	-	-	N/A
10	-	-	N/A

Anti-Ro/SS-A positivity was present in all 10 patients with normal-pressure glaucoma, using hospital-based ELISA. One of the patients had an elevated erythrocyte sedimentation rate, and one had an elevated rheumatoid factor titer in her sera. Four patients had antinuclear antibody titers of more than 1:80, including a titer of 1:1,280 in one patient. Elevated circulating immune complex binding levels ((C.sub.1q)) were found in five patients (three modestly and two minimally). Antibodies to other extractable nuclear antigens (anti-ribonuclear protein, anti-Smith antigen) were present in one patient, and one patient had a monoclonal gammopathy (IgG (Kappa)). Ouchterlony immunodiffusion disclosed that one patient had anti-Ro precipitin (Table 3).

TABLE 3. Laboratory Findings of Patients with Normal-pressure Glaucoma and Anti-Ro/SS-A Positivity

Patient No.	ESR	ANA	RIF	(C.sub.1q)	C	
1	12	-	-	6 (*)	198	
3	N/A	-	-	25 (*)	255 (*)	
4	28 (*)	1:1,280	-	-	295 (*)	
5	N/A	-	1:160	-	299 (*)	
6	9	1:320	-	43 (*)	-	
7	6	1:160	-	-	216	
8	18	1:40	-	23 (*)	-	
9	1	-	-	6 (*)	207	
10	1	1:160	-	-	271 (*)	
Patient No.	(C.sub.3)	(C.sub.4)	Anti-SS-A	Anti-SS-B	PA	
1	142	50.1 (*)	+	-	-	
2	122	21.5	+	-	-	
3	99	18.9	+	-	-	
4	144	51.5 (*)	+	-	-	
5	113	37.9	+	-	-	
6	123	28.3	+	-	-	Ro
7	125	26.4	+	-	-	
8	153	39.8 (*)	+	+	-	
9	86	11.6 (*)	+	-	-	
10	110	33.6	+	+	-	
Patient No.	ENA	ACA	LA	CG	Monoclonal Gammopathy	

1	-	-	-	-	-
2	-	-	-	-	-
3	-	-	-	-	-
4	-	-	-	-	-
5	-	-	-	-	-
6	-	IgG	-	-	-
7	-	-	-	-	-
8	-	-	-	-	IgG (Kappa)
9	-	-	-	-	-
10	-	-	-	-	-

ESR = erythrocyte sedimentation rate (mm/hr); ANA = antinuclear antibody (quantitative titer); RF = rheumatoid factor (quantitative titer); (C.sub.1q) = immune complex; C = total complement (U/ml); (C.sub.3), (C.sub.4) (mg%); anti-SS-A = anti-Sjogren syndrome-A antibody (ELISA); anti-SS-B = anti-Sjogren syndrome-B antibody (ELISA); PA = precipitating antibodies to soluble tissue antigens (Ouchterlony immunodiffusion); ENA = other antibodies to extractable nuclear antigens; ACA = anticardiolipin; LA = lupus anticoagulant factor; CG = cryoglobulin.

(*) Abnormal levels.

Immunoblotting was performed using the sera of 10 patients with normal-pressure glaucoma and anti-Ro/SS-A positivity (by ELISA). The immunoblots using purified human recombinant Ro/SS-A antigen are shown in Figure 2. Only two of 10 patients with normal-pressure glaucoma (lanes 4 and 5) were weakly positive by western blotting for anti-Ro/SS-A antibodies, as demonstrated by a visible band at 60 kd. Based on our findings that most of our patients with normal-pressure glaucoma did not have immunoreactive sera containing anti-Ro/SS-A antibodies by western blotting (8/10) or Ouchterlony immunodiffusion (9/10), we reasoned that the patients who were positive for anti-Ro/SS-A by ELISA were positive because of either a false-positive result or low positive anti-Ro/SS-A antibody levels. We therefore sought to determine whether these patients had serum antibodies to other proteins that may have cross-reacted with Ro/SS-A antigen and thereby possibly account for these anti-Ro/SS-A findings.

(Figure 2 ILLUSTRATION OMITTED)

We identified several proteins known to be present in neural tissues that migrate to 60 kd on Western blots and that therefore are candidates to demonstrate antigenic immunoreactivity to Ro/SS-A. Immunoblotting was performed utilizing two antigenically distinct species of purified, recombinant Ro/SS-A (with molecular masses of 52 kd and 60 kd, respectively) (12-14) as antigens and antibodies to calreticulin, a calcium-binding protein of the endoplasmic reticulum, (15) bacterial and human hsp60, and glutamic acid decarboxylase. As shown in Figure 3, a markedly positive immunoreactivity against Ro/SS-A (60 kd) occurred with a rabbit polyclonal antiserum that recognizes both bacterial and human hsp60. Reactivity was also seen with calreticulin antiserum, and no reactivity was seen with a mouse monoclonal antibody against human hsp60 (MabII-13) or glutamic acid decarboxylase antiserum.

(Figure 3 ILLUSTRATION OMITTED)

Because the polyclonal antibody recognizes both

bacterial and human hsp60, further studies were performed to examine the cross-reactivity of a monoclonal antibacterial hsp60 antibody to human Ro/SS-A antigens. In Figure 3, bottom, monoclonal antibodies to bacterial hsp60 (anti-GroEL) recognized both 52 kd and 60 kd Ro/SS-A antigens, as shown by immunoreactive bands at 52 kd and 60 kd, respectively. Furthermore, antisera to human Ro/SS-A demonstrated marked immunoreactivity to bacterial hsp60 but not to human hsp60.

To summarize, we have shown at least two antibodies that bind to hsp60 epitopes (both bacterial and human) and that also strongly bind to human Ro/SS-A. Because of this, we then sought to determine whether all of the anti-Ro/SS-A-positive (by ELISA) sera from patients with normal-pressure glaucoma contained immunoreactivity against bacterial or human hsp60, or against both. The results of immunoblotting using the sera of these patients against both purified bacterial and human hsp60 are shown in Figure 4. Indeed, nine of the 10 patients demonstrated marked immunoreactivity in their sera against bacterial hsp60, whereas five of the patients were positive against human hsp60.

(Figure 4 ILLUSTRATION OMITTED)

Examination of the Western blots using sera of 10 patients with normal-pressure glaucoma and Ro/SS-A positivity (by ELISA) disclosed IgG antibodies against a 60 kd protein in the soluble fraction of bovine retina in all of the patients. Figure 5 demonstrates the Western blot findings of two representative patients. Each lane contains bovine retinal supernatant, purified bacterial hsp60, or human hsp60, as labeled. Immunoreactivity, as visualized by a band in the region of 60 kd, can readily be seen in the lanes against the retinal supernatant and bacterial hsp60. Because the patient sera recognized bacterial hsp60 and a protein present in retinal supernatant, we were interested in testing whether this retinal protein is immunologically related to bacterial hsp60. If so, we would then predict that adsorbing retinal supernatant to a column of immobilized hsp60 antibodies should specifically deplete the supernatant of the 60 kd band. Figure 6 shows the results of immunoblotting using sera from two patients and bovine retina supernatant that was preadsorbed with monoclonal antibody to human hsp60 bound to sepharose. In each patient, the 60 kd band present in retinal supernatants is no longer visualized following adsorption.

(Figures 5 & 6 ILLUSTRATION OMITTED)

The results of the ELISA for bacterial and human hsp60 in patients with glaucoma were as follows. The observation that increased serum immunoreactivity to bacterial hsp60 was found in nine of our 10 anti-Ro/SS-A-positive (by ELISA) patients prompted us to inquire whether this finding would be meaningful when the sera from a larger cohort of patients with normal-pressure glaucoma (78 patients; mean age, 71.3 (+ or -) 12.0 years) was examined in comparison with sera from patients with primary open-angle glaucoma (22 patients; mean age, 72.2 (+ or -) 12.0 years) and from

nonglaucomatous control subjects (29 subjects; mean age, 71.0 (+ or -) 12.0 years). We therefore performed an ELISA on these patient groups. The diagnostic criteria for the patients with primary open-angle glaucoma were similar to those of the patients with normal-pressure glaucoma except that the intraocular pressure levels of the patients with open-angle glaucoma were greater than 23 mm Hg, as previously described.(2) The results of ELISA, using both bacterial and human hsp60, are shown in Table 4. Immunoreactivities against both bacterial and human hsp60 were higher in the patients with normal-pressure glaucoma than those in the control patients (Mann-Whitney U test, P (is less than) .0001 and P (is less than) .0001, respectively). Immunoreactivity of the patients with normal-pressure glaucoma against bacterial hsp60 was also higher than

was

that in the patients with primary open-angle glaucoma (Mann-Whitney U test, P = .04). However, serum immunoreactivities against human hsp60 were similar in the patients with normal-pressure glaucoma and primary open-angle glaucoma (Mann-Whitney U test, P = .09).

TABLE 4. ELISA Results for Anti-hsp60

Variable	Anti-bacterial hsp60(*)	Anti-human hsp60(*)
Patient group		
NPG (n = 78)	0.17 (+ or -) 0.2	0.18 (+ or -) 0.1
POAG (n = 22)	0.09 (+ or -) 0.08	0.12 (+ or -) 0.1
Control (n = 29)	0.05 (+ or -) 0.06	0.02 (+ or -) 0.04
P value((dagger))		
NPG vs POAG	.04	.09
NPG vs Control	<.0001	<.0001
POAG vs Control	.05	.0001

NPG = normal-pressure glaucoma; POAG = primary open-angle glaucoma.

(*) Optic density readings (mean (+ or -) SD).

((dagger)) Based on the Mann-Whitney U test.

We also compared the percentage of patients in three groups who had higher ELISA titers than the mean level for all three groups. The percentage of patients with a high titer of serum immunoreactivity against bacterial hsp60 was higher in the normal-pressure glaucoma group (41%) than the percentage in the primary open-angle glaucoma (14%) and control (3%) groups (Fisher exact test, P = .02 and P = .0002, respectively). Similarly, the percentage of patients with a high titer of immunoreactivity against human hsp60 in the normal-pressure glaucoma group (46%) was higher than that of the control patients (3%) (Fisher exact test, P (is less than) .0001). However, the percentages of high titer of serum immunoreactivity against human hsp60 were similar in the groups with normal-pressure glaucoma (46%) and primary open-angle glaucoma (32%) (Fisher exact test, P = .23).

DISCUSSION

There are no universally accepted diagnostic criteria for Sjogren syndrome, a clinical syndrome in which there is an association of dry eye (keratoconjunctivitis sicca), dry mouth, and rheumatoid arthritis. There are at least four diagnostic "schools" that have varying inclusion criteria for Sjogren syndrome.(16)

Although only one school requires the presence of serum antibodies against Ro/SS-A, all four require the presence of objectively abnormal ocular tests (Schirmer or rose bengal staining) and lymphocyte infiltration around the labial salivary glands in biopsy specimens. Although all of our 10 patients with normal-pressure glaucoma indeed had anti-Ro/SS-A positivity (by ELISA), we found it curious that none of our patients fulfilled objective criteria of Sjogren syndrome or other connective tissue disorders. In addition, the positive Schirmer test in four of the patients with normal slit-lamp findings could not be considered specific findings because dry eyes are a common finding in the elderly, and, in addition, dry eyes have been associated with the treatment of topical beta-blockers. (17,18) Our impression that clinical evidence of Sjogren syndrome was lacking in these patients was confirmed by rheumatologic consultation.

The exact biologic functions of the Ro and La ribonucleoproteins are unknown, although the roles of these proteins as important targets in autoimmune responses have been known since their first description by Anderson and associates in 1960. (19) Antibodies to ribonuclear particles, Ro/SS-A and La/SS-B, have been presented as a frequent feature of the autoimmune response in the sera of 70% to 90% of patients with Sjogren syndrome. Harley and associates, (20) using solid-phase assays (ELISA), reported that more than 96% of patients had anti-Ro/SS-A antibodies and 87% had anti-La/SS-B antibodies. However, these antibodies appear to be relatively nonspecific because they are also detected in the sera of patients with systemic lupus erythematosus and a variety of other connective tissue diseases. (21-23) In addition, low levels of these autoantibodies have been found in normal donors (24,25) and in the elderly. (26,27) The antigenic stimulus for the induction of Ro/SS-A is unknown, although several cross-reactivities of anti-Ro/SS-A antibodies as well as their concomitant specificities in anti-Ro/SS-A patient sera (28,31) have been demonstrated.

Because of the absence of clinical criteria for Sjogren syndrome in our 10 patients with normal-pressure glaucoma, it is possible that the presence of anti-Ro/SS-A antibodies (by ELISA) in these patients was somehow anomalous, or at the minimum represented non-organ-specific findings. The negative results of Western blotting against purified Ro antigens and the negative precipitin reactions of Ouchterlony immunodiffusion in essentially all (9/10) of our patients further suggest either that the positive results of hospital-based ELISA for anti-Ro/SS-A represent false-positive responses or that the level of anti-Ro/SS-A in these patients is detectable but low. These results, obtained by a commercially available ELISA, are consistent with data suggesting that solid-phase assays (ELISA) using purified antigens to assess anti-Ro and anti-La are more sensitive (at least 100-fold) than traditional Ouchterlony immunodiffusion methods are, although the latter have

higher specificity. (32,33) Also, patients with low levels of anti-Ro/SS-A autoantibodies often do not bind recombinant Ro/SS-A protein in Western blotting. This is because anti-Ro binding sites may be destroyed by the denaturation during antigen preparation for Western blotting; therefore, recombinantly expressed Ro protein may have a low level of antigenicity. (34,35)

Our hypothesis is that sera from these patients contain an antibody strongly active against a distinct antigen that may account for the results of the laboratory tests. To identify a putative autoantigen that might account for our false-positive findings, we initiated a series of experiments in which we examined the binding of several immunogens found in neural tissues, with Ro/SS-A. One of the autoantigens examined was calreticulin. Calreticulin is a (Ca.sup.++)-binding/storage protein in nonmuscle endoplasmic reticulum, especially in neuronal tissues, including retina, and has been claimed as a component of the Ro/SS-A system. (15) Although we found

cross-reactivity of calreticulin antibodies with Ro/SS-A, further experiments failed to disclose any positive immunoreactivity against purified calreticulin in the sera of our patients with normal-pressure glaucoma (results not shown).

Heat shock proteins serve as members of a diverse family of cellular "chaperonins"; their chaperonin function is derived from their role in intracellular folding (into oligomeric complexes) and assembly of other polypeptides. They are especially important during neuronal differentiation and neurite outgrowth, when much new cytoskeletal protein synthesis and polymer remodeling occurs. (36-38) They are also the major antigenic proteins of numerous pathogenic bacteria. The high degree of sequence conservation between prokaryotic and eukaryotic chaperonins makes them candidate antigens for models of autoimmunity based on molecular mimicry. (8) It has been postulated that the immune response to chaperonins has both protective and pathogenic potential. Healthy individuals may have the capacity to respond to these self-stress protein determinants in a manner that could help eliminate their own cells that are infected, transformed, or otherwise stressed by heat shock, nutrient deprivation, oxygen radicals, or metabolic disruption. The immune system may also use changes in expression of self-heat shock proteins as a signal for the detection and elimination of abnormal, malignant, or infected cells. (39,40) The detailed mechanisms involved have not been elucidated, nor has it been clearly established whether the immune response to chaperonins is a cause or a consequence of the autoimmune disease. (41)

Nine of our 10 patients reacted with bacterial hsp60. The relation between these antibodies and the binding of Ro/SS-A in the ELISA is very curious. Given the available data, one could argue that ELISA is providing falsepositive results in these subjects. This possibility cannot be distinguished with confidence from the possibility that anti-Ro/SS-A and anti-bacterial hsp60 are independent

antibody systems in these patients. The possibility of a crossreaction is consistent with the apparently crossreacted monoclonal antibodies (Figure 3). Also, there may be sufficient amino acid sequence similarity (higher than 50% for some stretches of 10 amino acids) (42,43) for antigenic cross-reactivity to occur between human Ro (60 kd) and bacterial hsp60.

We wish to emphasize that although there is some latitude for the interpretation of our results, the value of our observation appears to be twofold. First, the clinician's level of suspicion should be sufficiently raised by a positive anti-Ro/SS-A result by ELISA in patients with normal-pressure glaucoma, and that finding should be confirmed by more specific methods, such as Ouchterlony immunodiffusion. A negative finding with the latter, more specific test may avert costly rheumatologic evaluation and testing in these patients. Second, we believe that a positive anti-Ro/SS-A in these patients may signify a high level of serum immunoreactivity to bacterial hsp60 and thus be an important clue that their glaucomatous optic neuropathy may involve an as yet unidentified autoimmune mechanism.

Autoantigens that have sequence homology to bacterial hsp60 or demonstrate marked cross-reactivity to bacterial hsp60 are associated with numerous autoimmune diseases, including insulin-dependent juvenile diabetes (glutamic acid decarboxylase), systemic lupus erythematosus (DNA-binding protein, hsp90), scleroderma (Ku autoirrimune antigen), and multiple sclerosis (myelin-associated protein). (42,43) Based on the presence and strong association of hsp60 antibodies with these other immune-mediated diseases, our finding that immunoreactivity to bacterial hsp60 is significantly elevated in the sera of some patients with glaucoma, particularly with normal, pressure glaucoma, is consistent with the possibility that the origin of the glaucomatous optic neuropathy in some patients may involve an autoimmune mechanism triggered by an aberrant immune response to bacterial hsp60 in these patients. On the other hand, the increased immunoreactivity to human hsp60 in patients with normal-pressure glaucoma and in those with primary open-angle glaucoma may signify a common mechanism that underlies the optic neuropathy or may be a common phenomenon of glaucomatous damage in these patients and deserves further investigation.

It is difficult to determine whether the retinal arteriolar narrowing and nerve fiber layer hemorrhages in patients with normal-pressure glaucoma and autoantibodies may be signs of unique microvascular involvement in these patients. Although the presence of anti-Ro/SS-A positivity has been defined as a subset of patients with Sjogren syndrome who have systemic manifestations of vasculitis, (44,45) this association may be irrelevant in our patients because only one clearly has antibodies to Ro/SSA. However, the presence of antibodies against bacterial hsp60 may have clinical importance in these patients because vascular depositions of hsp60 were observed in vasculitis in Reiter disease. (46) A possible association between normal-pressure glaucoma and vasospasm (47) and, in turn, the occurrence of vasospasm in some forms

of immune-related disease is possibly relevant for those of our patients who had retinal arteriolar narrowing and nerve fiber layer hemorrhages.(48,49) If there is a structural disease or obstruction of the blood vessels by high blood viscosity or vasospasm induced by blood-borne immune complexes, as in many of the autoimmune diseases, then ordinary vasoconstriction could lead to critical reduction of the circulation and induce ischemia in these patients.

In summary, we believe that the presence of increased

autoantibodies to bacterial hsp60 in the sera of patients with normal-pressure glaucoma supports the hypothesis that glaucomatous optic neuropathy in a cohort of patients with normal-pressure glaucoma may involve aberrant autoimmunity. Furthermore, the identification of autoantibodies that react with human hsp60 in patients with normal-pressure and primary open-angle glaucoma may signify a common finding associated with the glaucomatous optic neuropathy process in some patients and appears to be unrelated to intraocular pressure levels. Further biochemical and molecular characterization of the components associated with antibodies toward antigens of retina or optic nerve, or both, in glaucomatous patients--in particular, those with normal-pressure glaucoma--may lead to a better understanding of the pathogenesis of this disease.

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Heat shock protein-peptide complexes: cancer vaccines with a new premise (Meeting abstract).

Srivastava
Fordham Univ.

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Our work has shown that homogeneous preparations of heat shock proteins (HSPs) derived from any cell type contain a wide assortment of peptides (6-35 mers) non-covalently bound to or 'chaperoned' by the HSP. The binding of peptides to HSPs occurs during the normal physiological degradation of proteins. One consequence of this phenomenon is that HSP preparations contain the entire repertoire of peptides generated in a cell. The repertoire consists of self peptides and antigenic peptides: thus, HSPs derived from tumors are complexed with peptides derived from tumor antigens and HSP preparations derived from virus-infected cells contain virus encoded peptides. Interestingly, vaccination of animals with such

HSP-peptide complexes elicits CD8+ T-cells specific for the antigenic peptides present in the vaccinating HSP preparation. In the case of cancers, this has been shown to result in protective immunity. This phenomenon appears to be general in that all three of the major cellular HSPs - hsp70, hsp90 and gp96 have been shown to act as cancer vaccines against three antigenically distinct mouse sarcomas, one rat hepatocarcinoma, two spindle cell mouse carcinomas and one mouse leukemia. Three points of interest emerge from the above: firstly, vaccination with HSP-peptide complexes consistently elicits protection from a tumor challenge, without necessarily eliciting a CTL response, as in chemically induced mouse sarcomas. However, in vivo depletion studies indicate that such vaccination consistently elicits a CD8+ response in vivo even when a CTL response is not detected in vitro. Secondly, vaccination with HSP-peptide complexes can be used to elicit protective cancer immunity without any prior knowledge of the epitopes recognized by CD8+ T-cells. This becomes possible because HSP preparations contain the entire repertoire of antigenic peptides of the cancer or other cells from which they are purified. Finally, vaccination with HSP-peptide complexes, like vaccination with intact tumors, elicits tumor-specific (as opposed to cross-reactive) protection. This must be viewed in the context of the contrasting fact that shared antigens can be detected on these tumors through cloned helper T-cells and CTLs. Our observations lead us to two premises. One, that CD8+ T-cells, rather than CTLs, play a significant cancer-protective role and that sole reliance on CTLs may be misleading. A distinction is made between CD8+ T-cells and CTLs, in that the latter are a category of the former. The fact that the two terms are often used interchangeably reflects our inability to measure any kind of CD8+ activity other than cytotoxicity, degranulation or cytokine release. This experimental limitation has begun to define our entire view of cellular immunity to cancer. The second premise is that cancers elicit a limited immune response to a broad array of epitopes, rather than a powerful response to a small number. This premise derives from the observation that vaccination with HSP-peptide complexes or with intact tumors elicits tumor-specific immunity. It is our belief that this uniqueness derives not from single antigens but from a large mosaic of randomly altered molecules, each of which are not very antigenic by themselves. This belief is clearly at odds with the current dominant paradigm of a small number of powerful shared melanoma antigens. The current paradigm rests on the observation that such antigens are the only kind detected by CTLs and derives strength from a solid body of data in viral immunity. Thus, the use of HSP-peptide or chaperone-peptide complexes for vaccination against cancer rests on a new set of premises, which are actually derived from the oldest observations in cancer immunity. The unique advantages of chaperone-based vaccination may be listed as follows: it permits vaccination against cancers without requiring prior determination of the antigenic epitopes of cancer cells; by virtue of association of HSPs with the entire repertoire of cellular peptides, such complexes are inherently multivalent and thus a (ABSTRACT TRUNCATED)

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Prevention of Mucosal Escherichia coli Infection by FimH-Adhesin-Based
Systemic Vaccination

Langermann, Solomon; Palaszynski, Susan; Barnhart, Michelle; Auguste, Gale; Pinkner, Jerome S.; Burlein, Jeanne; Barren, Philip; Koenig, Scott; Leath, Simon; Jones, C. Hal; Hultgren, Scott J.

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...Text: to type 1-piliated E. coli. Antibody was raised in mice to two forms of purified FimH protein: (i) a complex containing the periplasmic chaperone FimC bound to the full-length FimH protein (FimC-H), and (ii) a naturally occurring...

...FimHt), corresponding to the NH.inf(2)-terminal two-thirds of the FimH protein, was purified away from the FimC-H complex (B8) . Antibody was also raised to whole type 1...immunoglobulin G (IgG) titers to (A) FimHt adhesin and (B) whole type 1 pili after immunization with purified adhesin, adhesin- chaperone complex , or whole type 1 pili. The serum immune responses to FimH and whole type 1 pili were evaluated after primary immunization (day 0) [in complete Freund's adjuvant (CFA)] and booster immunization (week 4) [in incomplete Freund's adjuvant (IFA)] with purified FimHt (squares), FimC-H (circles), or whole type 1 pili (triangles) (B8) . The immunogenicity of...

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/CA96/00322 (22) International Filing Date: 17 May 1996 (17.05.96) (30) Priority Data: 08/472,534 7 June 1995 (07.06.95) US 60/001,805 4 August 1995 (04.08.95) US (71) Applicant (for all designated States except US): IAF BIOVAC INC. [CA/CA]; 525 Des Prairies Boulevard, Laval, Quebec H7N 4Z2 (CA). (72) Inventors; and (75) Inventors/Applicants (for US only): HAMEL, Josée [CA/CA]; (CA). BRODEUR, Bernard [CA/CA]; 2401 Maritain Street, Sillery, Quebec G1T 1N6 (CA). MARTIN, Denis [CA/CA]; 4728-G Gaboury Street, St-Augustin-de-DesMaures, Que- bec G3A 2X1 (CA). RIOUX, Clément [CA/CA]; 1012 Jean Charles Cantin, Ville de Cap-Rouge, Quebec G1Y 2X1 (CA). (74) Agents: DUBUC, Jean, Y. et al.; The Stock Exchange Tower, Suite 3400, 800 Square Victoria, P.O. Box 242, Montreal, Quebec H4Z 1E9 (CA).		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published With international search report.	

(54) Title: STREPTOCOCCAL HEAT SHOCK PROTEINS MEMBERS OF THE HSP70 FAMILY

(57) Abstract

Novel heat shock proteins (HSPs) of *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Streptococcus agalactiae* having apparent molecular masses of 70-72 kDa, immunologically related polypeptides, the nucleotide and derived amino acid sequences of HSP72 of *S. pneumoniae* (SEQ ID NO:4; SEQ ID NO:5), the nucleotide and derived amino acid sequences of HSP70 of *S. pyogenes* (SEQ ID NO:19; SEQ ID NO:20), the nucleotide and derived amino acid sequences of HSP 70 of *S. agalactiae* (SEQ ID NO:21; SEQ ID NO:22), antibodies that bind to the HSPs, and recombinant DNA methods for the production of the HSPs and immunologically related polypeptides are described. The polypeptides, DNA sequences and antibodies of this invention provide new means for the diagnosis, prevention and/or treatment of Streptococcal disease.

Considered

(Bacterial Vaccines)

Record Date Created: 19970918

Record Date Completed: 19970918

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DIALOG(R) File 155:MEDLINE(R)

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12875045 PMID: 10816527

Lack of protection in mice and necrotizing bronchointerstitial pneumonia with bronchiolitis in guinea pigs immunized with vaccines directed against the hsp60 molecule of *Mycobacterium tuberculosis*.

Turner O C; Roberts A D; Frank A A; Phalen S W; McMurray D M; Content J; Denis O; D'Souza S; Tanghe A; Huygen K; Orme I M

Mycobacteria Research Laboratories, Departments of Microbiology, Colorado State University, Fort Collins, Colorado, USA.

Infection and immunity (UNITED STATES) Jun 2000, 68 (6) p3674-9, ISSN 0019-9567 Journal Code: 0246127

Contract/Grant No.: AI-40488; AI; NIAID

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Document type: Journal Article

Languages: ENGLISH

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Subfile: INDEX MEDICUS

In this study, the hsp60 and hsp70 heat shock protein antigens of *Mycobacterium tuberculosis* were tested as potential vaccine candidates, using purified recombinant protein antigens or antigens encoded in the form of a DNA plasmid vaccine. Guinea pigs vaccinated with a mixture of the two proteins showed no evidence of resistance to low-dose aerosol challenge infection and quickly developed severe lung damage characterized by necrotizing bronchointerstitial pneumonia and bronchiolitis. As a result, we turned instead to a DNA vaccination approach using a plasmid encoding the hsp60 antigen of *M. tuberculosis*. Although immunogenic in mice vaccination with plasmid DNA encoding hsp60 was not protective in that model or in the guinea pig model and again gave rise to similar severe lung damage. This study seriously questions the safety of vaccines against tuberculosis that target highly conserved heat shock proteins.

Tags: Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, P.H.S.

Descriptors: *BCG Vaccine--therapeutic use--TU; *Chaperonin 60 --therapeutic use--TU; *Lung--pathology--PA; *Mycobacterium tuberculosis --immunology--IM; *Tuberculosis, Pulmonary--prevention and control--PC; Animals; Bronchiolitis--pathology--PA; Guinea Pigs; Mice; Necrosis; Pneumonia, Bacterial--pathology--PA; Vaccination; Vaccines, DNA --therapeutic use--TU

CAS Registry No.: 0 (BCG Vaccine); 0 (Chaperonin 60); 0 (Vaccines, DNA)

Record Date Created: 20000623

Record Date Completed: 20000623

25/9/39

DIALOG(R) File 155:MEDLINE(R)

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13130274 PMID: 11106940

Natural autoantibodies against heat-shock proteins hsp70 and gp96: implications for immunotherapy using heat-shock proteins.

4259279 PMID: 12065519

Cellular and molecular regulation of vaccination with heat shock protein 60 from *Histoplasma capsulatum*.

Deepe George S; Gibbons Reta S

Veterans Affairs Hospital and Division of Infectious Diseases, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267-0560, USA.
george.deepe@uc.edu

Infection and immunity (United States) Jul 2002, 70 (7) p3759-67,
ISSN 0019-9567 Journal Code: 0246127

Contract/Grant No.: AI-34361; AI; NIAID; AI-42737; AI; NIAID

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Languages: ENGLISH

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Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Vaccination with heat shock protein 60 (Hsp60) from *Histoplasma capsulatum* induces a protective immune response in mice. We explored the cellular and molecular requirements for the efficacy of recombinant Hsp60 in mice. Depletion of CD4(+), but not CD8(+), cells during the inductive phase of vaccination abolished protection, as assessed by survival and by the fungal burden in lungs and spleens. In the expressive phase, the elimination of CD4(+) or CD8(+) cells after immunization did not significantly alter fungal recovery or survival from a lethal challenge. Depletion of both subpopulations after Hsp60 vaccination resulted in a failure to control a lethal infection and a higher fungal burden in lungs and spleens. Cytokine release by spleen cells from mice vaccinated with Hsp60 produced substantially more gamma interferon and interleukin-10 and -12 than that of cells from mice immunized with either *H. capsulatum* recombinant Hsp70 or bovine serum albumin. The generation of gamma interferon, but not of interleukin-10, was dependent on T cells, in particular CD4(+) cells. Treatment of Hsp60-immunized mice with monoclonal antibody to gamma interferon or interleukin-10 or -12 in the inductive phase of vaccination was accompanied by increased recovery of yeast cells from lungs and spleens and 100% mortality. Likewise, the neutralization of gamma interferon or interleukin-12 abolished the protective effect of Hsp60 in the expressive phase. These results delineate the complexity of the regulatory elements necessary for vaccination against this fungus.

Tags: Research Support, U.S. Gov'

those providing the tumor antigens, we showed that hsp70 exerts efficacious adjuvant effects toward DC cross-priming. Hsp70 induces DC maturation and phagocytosis of cellular debris both in vitro and in vivo, which are conducive to CTL response to chaperoned and nonchaperoned antigens. Whereas the ability of hsp70 to induce cross-presentation of chaperoned peptides is natural killer (NK) independent, the adjuvant activity requires NK cells at the site of DC- hsp70 interaction to induce CTL response and therapeutic effect against lung metastases. However, although bystander activity provides equal CTL induction, the best therapeutic efficacy rests on cell vaccine secreting hsp70 that combines chaperoned antigen and danger signal within the same cell.

Record Date Created: 20050905

25/9/49

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11430107 PMID: 8757820

Immunity against Yersinia enterocolitica by vaccination with Yersinia HSP60 immunostimulating complexes or Yersinia HSP60 plus interleukin-12.

Noll A; Autenrieth IB

Institut fur Hygiene und Mikrobiologie der Universitat Wurzburg, Germany.

Infection and immunity (UNITED STATES) Aug 1996, 64 (8) p2955-61,

ISSN 0019-9567 Journal Code: 0246127

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

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Microbial heat shock proteins (HSP) are dominant antigens for the host immune response. Because of the high sequence homology between mammalian and microbial HSP, their value as component of a subunit vaccine has been the subject of controversy. Previous work from this laboratory, however, demonstrated for the first time that the adoptive transfer of HSP60-reactive CD4+ alphabeta T-cell clones confers protection against bacterial infection in mice but does not induce autoimmunity. In the present study, we have therefore evaluated the potential role of Yersinia HSP60 (Y- HSP60) as a vaccine in the Yersinia enterocolitica mouse infection model. For this purpose, immunostimulating complexes (ISCOM) which included Y- HSP60 were constructed. Parenteral administration of this vaccine induced high Y- HSP60 -specific serum antibody responses as well as T-cell responses. This reaction was paralleled by immunity against a lethal challenge with Y. enterocolitica. In contrast, mucosal application of Y- HSP60 -ISCOM failed to induce systemic Y- HSP60 -specific T-cell responses and thus failed to induce immunity against yersiniae. Likewise, vaccination with purified recombinant Y- HSP60 induced antibody responses but only weak T-cell responses. Therefore, this vaccination protocol was not protective. However, when interleukin-12 was used as an adjuvant, purified Y- HSP60 induced significant Y- HSP60 -specific T-cell responses and thus induced protection against subsequent challenge with yersiniae. These studies suggest that (i) microbial HSP might be promising candidates for the design of subunit vaccines and (ii) interleukin-12 is an efficient alternative adjuvant to ISCOM particles for induction of protective CD4 Th1-cell-dependent immune responses against bacterial pathogens.

Tags: Female; Research Support, Non-U.S. Gov't

Descriptors: *Chaperonin 60--therapeutic use--TU; *ISCOMs--therapeutic use--TU; *Interleukin-12--therapeutic use--TU; *Vaccination; *Yersinia

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4035471 PMID: 11803949

Induction of a th1-type of immune response but not protective immunity by intramuscular DNA immunisation with Brucella abortus GroEL heat-shock gene.

Leclercq Sophie; Harms Jerome S; Rosinha Gracia M S; Azevedo Vasco; Oliveira Sergio C

Department of Biochemistry and Immunology, Federal University of Minas Gerais, ICB-UFMG, Belo Horizonte-MG, Brazil.

Journal of medical microbiology (England) Jan 2002, 51 (1) p20-6,
ISSN 0022-2615 Journal Code: 0224131

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

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The immunogenicity and protective efficacy of a DNA vaccine encoding the GroEL heat-shock gene from Brucella abortus was tested in BALB/c mice immunised by intramuscular (i.m.) needle injection or epidermally by gene gun. The Brucella GroEL gene was amplified by PCR and cloned into two different mammalian expression vectors pCMV-link and pCMV-tPA. The D17 cell line was transfected with both constructs and GroEL transcripts were detected by Northern blot. To determine the level of protein synthesised, transfected cell lysates were then submitted to Western blot. The non-secreted form of the recombinant GroEL produced by the pCMV-link construct was detected in much greater amount than the secreted form of the protein produced by the pCMV-tPA construct. After immunisation, a strong anti-GroEL IgG response was detected in mice vaccinated by i.m. injection or gene gun only when the pCMV-link/ GroEL plasmid was used. Regarding the pattern of immune response induced, i.m. needle injection raised a predominantly Th1 response with mostly IgG2a-specific anti-GroEL and high levels of IFN-gamma produced by splenic T cells. Gene gun immunisation induced a Th0 type of immune response in mice characterised by a high IgG1/IgG2a ratio, and IL-4 and interferon (IFN)-gamma production. Even though a distinct pattern of immune response was generated depending upon the immunisation route used, neither method engendered a significant level of protection with the GroEL DNA vaccine.

Tags: Research Support, Non-U.S. Gov't

15451459 PMID: 15314192

Molecular cloning of the Chlamydomonas abortus groEL gene and evaluation of its protective efficacy in a murine model by genetic vaccination .

Hechard Celine; Grepinet Olivier; Rodolakis Annie

Unite de Pathologie Infectieuse et Immunologie, INRA-Centre de Tours, 37380 Nouzilly, France.

Journal of medical microbiology (England) Sep 2004, 53 (Pt 9) p861-8

, ISSN 0022-2615 Journal Code: 0224131

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

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The immunogenicity and protective effect of a DNA vaccine encoding the heat-shock protein (Hsp) GroEL of Chlamydomonas abortus AB7, an obligate intracellular bacterium that causes abortion in sheep, was evaluated in pregnant and non-pregnant mouse models. The C. abortus groEL gene was cloned by screening a genomic library constructed in lambdaFIX II arms with a nucleic acid probe corresponding to the central portion of the groEL gene from C. abortus. Sequence analysis of a positive clone revealed an open reading frame of 1632 bp encoding a 544 amino acid polypeptide with a predicted molecular mass of 58 256 Da and highly similar to GroEL of Chlamydia trachomatis (93 %) and Chlamydomonas pneumoniae (94 %). As observed in other sequenced chlamydial genomes, the groEL gene belongs to an operon comprising another gene encoding the Hsp GroES . OF1 outbred mice were immunized intramuscularly with plasmid DNA carrying the groEL gene three times at 3 week intervals and challenged 2 weeks after the last DNA injection. In pregnant mice, no reduction in abortion was observed and the DNA vaccination failed to reduce the bacterial infection in the placenta and spleen of mice. Nevertheless, partial protection of fetuses was obtained. Immunization of non-pregnant mice with the groEL gene resulted in a specific humoral response with the predominant IgG2a isotype, suggesting a Th1-type immune response. The anti-GroEL antibodies showed no neutralizing effect in vitro on C. abortus infectivity. Although the DNA vaccine induced a delayed-type hypersensitivity response, it failed to elicit an efficient cellular immune response since the mice were not protected against bacterial challenge.

Tags: Female; Pregnancy; Research Support, Non-U.S. Gov't

Descriptors: *Abortion, Spontaneous--prevention and control--PC; *Chlamydomonas Infections--prevention and control--PC; *Cloning, Molecular; * GroEL Protein --genetics--GE; *Pregnancy Complications, Infectious --prevention and control--PC; * Vaccines , DNA--immunology--IM; Animals; Animals, Outbred Strains; Bacterial Vaccines --administration and dosage --AD; Bacterial Vaccines --genetics--GE; Bacterial Vaccines --immunology--IM; Chlamydomonas--genetics--GE; Chlamydomonas--immunology --IM; Chlamydomonas Infections--microbiology--MI; GroEL Protein --immunology--IM; Mice; Molecular Sequence Data; Plasmids; Pregnancy; Sequence Analysis, DNA; Vaccines , DNA--administration and dosage--AD

Molecular Sequence Databank No.: GENBANK/AY052785

CAS Registry No.: 0 (Bacterial Vaccines); 0 (GroEL Protein); 0 (Plasmids); 0 (Vaccines, DNA)

Record Date Created: 20040817

Record Date Completed: 20041006

19/9/2

DIALOG(R) File 155:MEDLINE(R)

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16327169 PMID: 15544517

Adjunctive immunotherapy of mycobacterial infections.

Tomioka Haruaki

Department of Microbiology and Immunology, Faculty of Medicine, Shimane University, Izumo, Shimane 693-8501, Japan. tomioka@med.shimane-u.ac.jp

Current pharmaceutical design (Netherlands) 2004, 10 (26) p3297-312, ISSN 1381-6128 Journal Code: 9602487

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Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

In order to cope with the worldwide increase in the prevalence of multidrug-resistant tuberculosis and Mycobacterium avium complex (MAC) infections, a number of new antimycobacterial drugs have been or are being synthesized and developed. Development of new protocols for chemotherapy of refractory mycobacterioses is also sharing promise. In this context, one promising strategy is to devise regimens to treat patients with refractory mycobacterioses using ordinary antimycobacterial agents in combination with appropriate immunomodulators. This article deals with the following matters: an outline of the host immune response to mycobacterial pathogens, particularly in terms of mobilization of the cytokine network in response to mycobacterial infection, and adjunctive immunotherapy using (1) recombinant immunomodulating cytokines, (especially Th-1 and Th-1-like cytokines such as IFN-gamma, IL-2, IL-12, IL-18 and GM-CSF), (2) inhibitors of immunosuppressive cytokines (TGF-beta) and some proinflammatory tissue-damaging cytokines (TNF-alpha), and (3) immunomodulatory agents such as ATP and its analogs, imidazoquinoline, diethyldithiocarbamate, poloxamer, dibenzopyran, galactosylceramide, nonsteroidal anti-inflammatory drugs, Chinese traditional medicines, levamisole, synthesized mycobacterial oligoDNA, DNA vaccine expressing mycobacterial HSP65 or IL-12, and heat-killed Mycobacterium vaccae. Although adjunctive immunotherapy is fairly efficacious in treating intractable mycobacterioses, it still features serious problems and dilemmas, such as high cost, occasionally severe side effects, and, in many cases, only modest efficacy in potentiating host defense mechanisms against mycobacterial infections, primarily because of the induction of macrophage-deactivating cytokines during the course of long-term administration of adjunctive agents. (110 Refs.)

Descriptors: *Drug Therapy, Combination; *Immunotherapy--methods--MT; *Mycobacterium Infections--



US 20030099665A1

(19) **United States**

(12) **Patent Application Publication**

Langermann et al.

(10) **Pub. No.: US 2003/0099665 A1**

(43) **Pub. Date: May 29, 2003**

(54) **CHAPERONE AND ADHESIN PROTEINS;
VACCINES, DIAGNOSTICS AND METHOD
FOR TREATING INFECTIONS**

(76) **Inventors: Solomon Langermann, Baltimore, MD
(US); Scott J. Hultgren, Ballwin, MO
(US); Jerome S. Pinkner, St. Louis,
MO (US); Christine Gale Auguste,
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Roseland, NJ 07068 (US)**

(21) **Appl. No.: 10/288,978**

(22) **Filed: Nov. 6, 2002**

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(63) **Continuation of application No. 09/298,494, filed on
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(60) **Provisional application No. 60/082,824, filed on Apr.
23, 1998.**

Publication Classification

(51) **Int. Cl.⁷ A61K 39/40; A61K 39/02**

(52) **U.S. Cl. 424/190.1; 424/164.1**

(57) **ABSTRACT**

The present invention provides bacterial immunogenic agents for administration to humans and non-human animals to stimulate an immune response. It particularly relates to the vaccination of mammalian species with heteropolymeric protein complexes as a mechanism for stimulating production of antibodies that protect the vaccine recipient against infection by pathogenic bacterial species. In another aspect the invention provides antibodies against such proteins and protein complexes that may be used as diagnostics and/or as protective/treatment agents for pathogenic bacterial species. A novel vector for expressing the FimC-H complex at optimal levels is also disclosed.

DERWENT-ACC-NO: 2003-678130
DERWENT-WEEK: 200364
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TITLE: New vaccine comprising bacterial chaperone protein or bacterial adhesin protein FimH or a mannose-binding fragment of FimH, useful for preventing or treating enterobacterial infections

Basic Abstract Text (1):

NOVELTY - A vaccine against bacterial infections comprising a complex of a bacterial chaperone protein with an adhesin protein or an immunogenic fragment of the adhesin protein, is new.

Basic Abstract Text (3):

(1) a vaccine against bacterial infections comprising a complex of a bacterial chaperone protein with an adhesin protein or an immunogenic fragment of the adhesin protein;

Basic Abstract Text (12):

USE - The vaccine is useful for preventing or treating enterobacterial infections (claimed), particularly, the vaccine is used for urinary tract or bladder infections caused by Escherichia coli. The antibody raised against a complex of a bacterial chaperone protein is useful for detecting urinary tract infections and for the prevention and/or treatment of urinary tract infections caused by Escherichia coli (all claimed).

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•	<u>#1</u> Search austin helicobacter 1992	13:03:29	

Biochemistry. 1995 Nov 14;34(45):14918-31.

Related Articles, Links

Complexes between chaperonin GroEL and the capsid protein of bacteriophage HK97.

Ding Y, Duda RL, Hendrix RW, Rosenberg JM.

Department of Biological Sciences, University of Pittsburgh, Pennsylvania 15260, USA.

The 42 kDa capsid protein of bacteriophage HK97 requires the GroEL and GroES chaperonin proteins of its *Escherichia coli* host to facilitate correct folding, both in vivo and in vitro. In the absence of GroES and ATP, denatured gp5 forms a stable complex with the 14 subunit GroEL molecule. We characterized the electrophoretic and biochemical properties of this complex. In electrophoresis on a native (nondenaturing) gel, the band of the gp5-GroEL complex shifts to a slower migrating position relative to uncomplexed GroEL. The results show that there is only one subunit of gp5 bound to each GroEL 14-mer and that the shift in band position is due primarily to a change in the overall charge of the complex relative to uncomplexed GroEL, and not to a change in size or shape. GroEL forms similar complexes with proteolytic fragments of gp5, with a series of sequence duplication derivatives of gp5, and with other proteins. Electrophoretic examination of these complexes shows that a band shift occurs with proteins larger than 31-33 kDa but not with smaller proteins. For those proteins that cause a band shift upon complex formation, the magnitude of the shift is correlated with the predicted if the charge of the complex were simply the sum of the charge of GroEL and the charge of the substrate protein. We suggest that binding of a substrate protein to GroEL is accompanied by a net binding of solution cations to the complex, but only in the case of proteins above a minimum size of 31-33 kDa. The gp5-GroEL complex is in an association/dissociation equilibrium, with a binding constant measured in the range of 11-17 microM⁻¹.

PMID: 7578104 [PubMed - indexed for MEDLINE]

Eur J Immunol. 1991 Oct;21(10):2297-302.

[Related Articles, Links](#)**Mycobacterial heat-shock proteins as carrier molecules.****Lussow AR, Barrios C, van Embden J, Van der Zee R, Verdini AS, Pessi A, Louis JA, Lambert PH, Del Giudice G.**

World Health Organization-Immunology Research and Training Center, Department of Pathology, University of Geneva, Switzerland.

We have previously shown that the priming of mice with live *Mycobacterium tuberculosis* var. bovis (Bacillus Calmette-Guerin, BCG) and immunization with the repetitive malaria synthetic peptide (NANP)₄₀ conjugated to purified protein derivative (PPD), led to the induction of high and long-lasting titers of anti-peptide IgG antibodies, overcoming the requirement of adjuvants and the genetic restriction of the antibody response to the peptide (Lussow et al., Proc. Natl. Acad. Sci. USA 1990. 87:2960). This initial work led us to the following observations. BCG had to be live for priming to lead to the induction of anti-peptide antibodies. Surprisingly, priming with other living microorganisms which chronically infect the macrophage (e.g. *Salmonella typhimurium* and *Leishmania major*) also induced anti-peptide antibodies in mice immunized with PPD-(NANP)₄₀ conjugate. It was, thus, hypothesized that molecules expressed during active infection and also known to be highly conserved between species, namely the heat-shock proteins (hsp), could mediate the T cell sensitization required for the production of anti-peptide antibodies. In fact, when the PPD portion of the conjugate was replaced by a highly purified recombinant protein corresponding to the 65-kDa (GroEL-type) hsp of *M. bovis*, this resulted in the production of anti-(NANP) IgG antibodies in BCG-primed mice, irrespective of the major histocompatibility complex-controlled responsiveness to the (NANP) sequence itself. Further, similar induction of anti-peptide antibody response was also obtained with a recombinant 70-kDa (DnaK-type) hsp of *M. tuberculosis*, but not with a small molecular mass (18 kDa) of *M. leprae*. Finally, an adjuvant-free carrier effect for anti-peptide IgG antibody production in BCG-primed mice, was also exerted by the GroEL hsp of *Escherichia coli*. This finding that hsp can act as carrier molecules without requiring conventional adjuvants is of potential importance in the development of vaccine strategies.

PMID: 1680693 [PubMed - indexed for MEDLINE]

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File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec
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File 467:ExtraMED(tm) 2000/Dec
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***File 467: F467 no longer updates; see Help News467.**

Set	Items	Description
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?
? e chaperon

Ref	Items	RT	Index-term
E1	1		CHAPEROMINE
E2	3067		*CHAPERON
E3	1		CHAPERON CHAPERONE
E4	1		CHAPERON GENE
E5	1		CHAPERON GROE
E6	1		CHAPERON HSP90
E7	2		CHAPERON INDUCER
E8	1		CHAPERON INDUCING AGENT
E9	1		CHAPERON INHIBITOR
E10	2		CHAPERON MOLECULAIRE
E11	1128	4	CHAPERON PROTEIN
E12	1		CHAPERON PROTEIN CHAPERONE PROTEIN

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Ref	Items	Index-term
E13	3	CHAPERON PROTEINS
E14	1	CHAPERON ROUGE
E15	3	CHAPERON SOLVENT PLUG
E16	1	CHAPERON SOLVENT PLUG CHAPERONE SOLVENT PLUG
E17	1	CHAPERON SYSTEM CHAPERONE SYSTEM
E18	1	CHAPERON-ENCODING
E19	2	CHAPERON-LIKE ACTIVITY
E20	1	CHAPERON-LIKE EFFECT
E21	1	CHAPERON-LIKE INTERACTIONS
E22	1	CHAPERON-MEDIATED
E23	1	CHAPERON-PROTEINS
E24	19	CHAPERONAGE

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? s e2-e23

	3067	CHAPERON
	1	CHAPERON CHAPERONE
	1	CHAPERON GENE
	1	CHAPERON GROE
	1	CHAPERON HSP90
	2	CHAPERON INDUCER
	1	CHAPERON INDUCING AGENT
	1	CHAPERON INHIBITOR
	2	CHAPERON MOLECULAIRE
	1128	CHAPERON PROTEIN
	1	CHAPERON PROTEIN CHAPERONE PROTEIN
	3	CHAPERON PROTEINS
	1	CHAPERON ROUGE
	3	CHAPERON SOLVENT PLUG
	1	CHAPERON SOLVENT PLUG CHAPERONE SOLVENT PLUG
	1	CHAPERON SYSTEM CHAPERONE SYSTEM
	1	CHAPERON-ENCODING
	2	CHAPERON-LIKE ACTIVITY
	1	CHAPERON-LIKE EFFECT
	1	CHAPERON-LIKE INTERACTIONS
	1	CHAPERON-MEDIATED
	1	CHAPERON-PROTEINS
S1	3067	E2-E23

? e chaperonin

Ref	Items	RT	Index-term
E1	1		CHAPERONII
E2	15325	7	*CHAPERONIN
E3	1		CHAPERONIN --CLINICAL TRIAL --CT
E4	1		CHAPERONIN --DRUG ANALYSIS --AN
E5	1		CHAPERONIN --DRUG COMPARISON --CM
E6	5		CHAPERONIN --DRUG DEVELOPMENT --DV
E7	2		CHAPERONIN --DRUG THERAPY --DT
E8	2		CHAPERONIN --DRUG TOXICITY --TO
E9	769		CHAPERONIN --ENDOGENOUS COMPOUND --EC
E10	2		CHAPERONIN --INTRAMUSCULAR DRUG ADMINISTRATION
E11	1		CHAPERONIN --INTRAPERITONEAL DRUG ADMINISTRATI
E12	1		CHAPERONIN --ORAL DRUG ADMINISTRATION --PO

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Ref	Items	Index-term
E13	7	CHAPERONIN --PHARMACOLOGY --PD
E14	2	CHAPERONIN ACTION
E15	3	CHAPERONIN ACTIVITY
E16	1	CHAPERONIN APICAL DOMAIN
E17	3	CHAPERONIN ASSEMBLY
E18	1	CHAPERONIN ASSISTED FOLDING
E19	1	CHAPERONIN ASSISTED OVEREXPRESSION
E20	1	CHAPERONIN ASSISTED RENATURATION
E21	2	CHAPERONIN ATPASE
E22	20	CHAPERONIN ATPASE CYCLE
E23	1	CHAPERONIN BINDING DOMAIN
E24	1	CHAPERONIN BINDING INTERFERENCE

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E25	1	CHAPERONIN BINDING SITES
E26	1	CHAPERONIN BIP (78- KDA GLUCOSE-REGULATED PROT
E27	1	CHAPERONIN BIP (78-KDA GLUCOSE-REGULATED PROTE
E28	1	CHAPERONIN CAGE
E29	2	CHAPERONIN CAPTURE COMPLEX
E30	1	CHAPERONIN CAVITIES
E31	11	CHAPERONIN CCT
E32	1	CHAPERONIN CCT ANTIBODY
E33	1	CHAPERONIN CCT SUBUNIT GENE
E34	1	CHAPERONIN CCT SUBUNITS
E35	1	CHAPERONIN CCT-GAMMA
E36	1	CHAPERONIN CCTG GENE

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Ref	Items	Index-term
E37	1	CHAPERONIN CCT6 SUBUNIT
E38	6	CHAPERONIN COFACTOR A
E39	7	CHAPERONIN COMPLEX
E40	1	CHAPERONIN COMPLEX ARCHITECTURE
E41	1	CHAPERONIN COMPLEX ASSEMBLY
E42	1	CHAPERONIN COMPLEX COMPONENTS

E43	5	CHAPERONIN COMPLEXES
E44	1	CHAPERONIN CONTAINING
E45	1	CHAPERONIN CONTAINING T COMPLEX POLYPEPTIDE
E46	6	CHAPERONIN CONTAINING T COMPLEX POLYPEPTIDE 1
E47	1	CHAPERONIN CONTAINING T COMPLEX PROTEIN 1
E48	1	CHAPERONIN CONTAINING T-COMPLEX POLYPEPTIDE

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E49	10	CHAPERONIN CONTAINING T-COMPLEX POLYPEPTIDE 1
E50	3	CHAPERONIN CONTAINING T-COMPLEX POLYPEPTIDE-1

? s e1-e50

1	CHAPERONII
15325	CHAPERONIN
1	CHAPERONIN --CLINICAL TRIAL --CT
1	CHAPERONIN --DRUG ANALYSIS --AN
1	CHAPERONIN --DRUG COMPARISON --CM
5	CHAPERONIN --DRUG DEVELOPMENT --DV
2	CHAPERONIN --DRUG THERAPY --DT
2	CHAPERONIN --DRUG TOXICITY --TO
769	CHAPERONIN --ENDOGENOUS COMPOUND --EC
2	CHAPERONIN --INTRAMUSCULAR DRUG ADMINISTRATION
1	CHAPERONIN --INTRAPERITONEAL DRUG ADMINISTRATI
1	CHAPERONIN --ORAL DRUG ADMINISTRATION --PO
7	CHAPERONIN --PHARMACOLOGY --PD
2	CHAPERONIN ACTION
3	CHAPERONIN ACTIVITY
1	CHAPERONIN APICAL DOMAIN
3	CHAPERONIN ASSEMBLY
1	CHAPERONIN ASSISTED FOLDING
1	CHAPERONIN ASSISTED OVEREXPRESSION
1	CHAPERONIN ASSISTED RENATURATION
2	CHAPERONIN ATPASE
20	CHAPERONIN ATPASE CYCLE
1	CHAPERONIN BINDING DOMAIN
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1	CHAPERONIN CCT SUBUNIT GENE
1	CHAPERONIN CCT SUBUNITS
1	CHAPERONIN CCT-GAMMA
1	CHAPERONIN CCTG GENE
1	CHAPERONIN CCT6 SUBUNIT
6	CHAPERONIN COFACTOR A
7	CHAPERONIN COMPLEX
1	CHAPERONIN COMPLEX ARCHITECTURE
1	CHAPERONIN COMPLEX ASSEMBLY
1	CHAPERONIN COMPLEX COMPONENTS
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1	CHAPERONIN CONTAINING T COMPLEX POLYPEPTIDE
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1	CHAPERONIN CONTAINING T COMPLEX PROTEIN 1

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          10 CHAPERONIN CONTAINING T-COMPLEX POLYPEPTIDE 1
          3  CHAPERONIN CONTAINING T-COMPLEX POLYPEPTIDE-1
S2      15326  E1-E50
? e e2

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Ref  Items  Type  RT  Index-term
R1      1882      7  *CHAPERONIN
R2    394076      DC=D4.680
R3       42    B 5116  PEPTIDES AND PROTEINS
R4        0    S   1  CHAPERONIN 10
R5        0    S   1  CHAPERONIN 60
R6       427    S   1  CHAPERONINS
R7        0    S   1  GROEL PROTEIN
R8        0    S   1  GROES PROTEIN

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? s r1 or r2 or r4:r7

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>>> or undefined in one or more files.

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          1882  CHAPERONIN
          394076  DC=D4.680
          427  CHAPERONIN 10:GROEL PROTEIN
S3    394244  'CHAPERONIN' OR DC='D4.680' OR R4:R7

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? e chaperonin containing

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E2      5  CHAPERONIN COMPLEXES
E3      1  *CHAPERONIN CONTAINING
E4      1  CHAPERONIN CONTAINING T COMPLEX POLYPEPTIDE
E5      6  CHAPERONIN CONTAINING T COMPLEX POLYPEPTIDE 1
E6      1  CHAPERONIN CONTAINING T COMPLEX PROTEIN 1
E7      1  CHAPERONIN CONTAINING T-COMPLEX POLYPEPTIDE
E8     10  CHAPERONIN CONTAINING T-COMPLEX POLYPEPTIDE 1
E9      3  CHAPERONIN CONTAINING T-COMPLEX POLYPEPTIDE-1
E10     2  CHAPERONIN CONTAINING T-COMPLEX PROTEIN
E11     3  CHAPERONIN CONTAINING TAILLESS COMPLEX POLYPEP
E12     2  CHAPERONIN CONTAINING TAILLESS COMPLEX PROTEIN

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E13     1  CHAPERONIN CONTAINING TAILLESS POLYPEPTIDE-1
E14      3  CHAPERONIN CONTAINING TCP 1
E15      1  CHAPERONIN CONTAINING TCP 1 ALPHA
E16      1  CHAPERONIN CONTAINING TCP 1 DELTA
E17      1  CHAPERONIN CONTAINING TCP 1 EPSILON
E18      1  CHAPERONIN CONTAINING TCP 1 ETA
E19     17  CHAPERONIN CONTAINING TCP-1
E20      6  CHAPERONIN CONTAINING TCP-1 CCT
E21      1  CHAPERONIN CONTAINING TCP-1 COMPLEX
E22      2  CHAPERONIN CONTAINING TCP-1 GAMMA SUBUNIT (CCT
E23      2  CHAPERONIN CONTAINING TCP-1 RELATED SUBUNIT CH
E24      1  CHAPERONIN CONTAINING TCP-1-ACTIN COMPLEX

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S4          5  'CHAPERONIN COMPLEXES'

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Ref  Items  Index-term

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E25	1	CHAPERONIN CONTAINING TCP-1-GAMMA
E26	1	CHAPERONIN CONTAINING TCP-1-TUBULIN COMPLEX
E27	1	CHAPERONIN CONTAINING TCP1 CCT
E28	1	CHAPERONIN CONTAINING THE T-COMPLEX POLYPEPTID
E29	1	CHAPERONIN CONTAINING TUBULIN
E30	3	CHAPERONIN CPN60
E31	1	CHAPERONIN CPN60 1
E32	1	CHAPERONIN CPN60 2
E33	1	CHAPERONIN CPN60 3
E34	3	CHAPERONIN CYCLE
E35	1	CHAPERONIN CYCLING SPEED
E36	1	CHAPERONIN CYTOCHROME C RESPIRATORY CHAIN MITO

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? s e30:e33

S5 4 'CHAPERONIN CPN60': 'CHAPERONIN CPN60 3'

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Ref	Items	Index-term
E37	1	CHAPERONIN EVOLUTION
E38	1	CHAPERONIN EXPRESSION
E39	1	CHAPERONIN EXPRESSION PLASMID CONSTRUCTION
E40	1	CHAPERONIN FAMILY
E41	2	CHAPERONIN FILAMENTS
E42	6	CHAPERONIN FUNCTION
E43	1	CHAPERONIN FUNCTION BETA LACTAMASE
E44	1	CHAPERONIN FUNCTIONAL CYCLE
E45	5	CHAPERONIN GENE
E46	1	CHAPERONIN GENE (HOMINIDAE) CCT-2 GENE
E47	1	CHAPERONIN GENE COMPLEX
E48	1	CHAPERONIN GENE FAMILY

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Ref	Items	Index-term
E49	4	CHAPERONIN GENES
E50	1	CHAPERONIN GLUTAMATE AMMONIA LIGASE COMPLEX

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Ref	Items	Index-term
E1	1	CHAPERONIN GLUTAMATE AMMONIA LIGASE COMPLEX
E2	1	CHAPERONIN GRO-EL OVERPRODUCTION
E3	1	CHAPERONIN GRO-ES OVERPRODUCTION
E4	5	CHAPERONIN GROE
E5	1	CHAPERONIN GROE-FACILITATED REFOLDING
E6	161	CHAPERONIN GROEL
E7	1	CHAPERONIN GROEL MUTANT
E8	1	CHAPERONIN GROEL/ES GENES
E9	7	CHAPERONIN GROES
E10	1	CHAPERONIN HEMICYCLE
E11	1	CHAPERONIN HISTORY
E12	1	CHAPERONIN HOMOLOG

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? s e2 or e3

S6 1 CHAPERONIN GRO-EL OVERPRODUCTION
1 CHAPERONIN GRO-ES OVERPRODUCTION
1 'CHAPERONIN GRO-EL OVERPRODUCTION' OR 'CHAPERONIN GRO-ES OVERPRODUCTION'

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Ref	Items	Index-term
E13	4	CHAPERONIN HSP60
E14	1	CHAPERONIN HYBRIDS
E15	2	CHAPERONIN I
E16	2	CHAPERONIN II
E17	1	CHAPERONIN INTERACTION
E18	1	CHAPERONIN LOCALIZATION
E19	1	CHAPERONIN MACHINE
E20	1	CHAPERONIN MACHINERY
E21	1	CHAPERONIN MECHANISM
E22	1	CHAPERONIN MEDIATED FOLDING
E23	3	CHAPERONIN MOLECULE
E24	5	CHAPERONIN MOLECULES

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Ref	Items	Index-term
E25	2	CHAPERONIN MONOMER
E26	1	CHAPERONIN ORF 2
E27	1	CHAPERONIN OVEREXPRESSION
E28	1	CHAPERONIN PROPERTY
E29	8	CHAPERONIN PROTEIN
E30	1	CHAPERONIN PROTEIN CPN10
E31	1	CHAPERONIN PROTEIN FAMILY
E32	1	CHAPERONIN PROTEIN FOLDING INTERMEDIATE STABIL
E33	1	CHAPERONIN PROTEIN FOLDING INTERMEDIATE TRANSF
E34	1	CHAPERONIN PROTEIN GROEL
E35	4	CHAPERONIN PROTEIN HSP60
E36	1	CHAPERONIN PROTEIN SUBUNITS

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? s e29:e36

S7 17 'CHAPERONIN PROTEIN': 'CHAPERONIN PROTEIN SUBUNITS'

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Ref	Items	Index-term
E37	1	CHAPERONIN PROTEIN TEMPLATE
E38	11	CHAPERONIN PROTEINS
E39	2	CHAPERONIN PURIFICATION
E40	1	CHAPERONIN REACTION CYCLE
E41	1	CHAPERONIN REGULATOR
E42	1	CHAPERONIN REQUIREMENT
E43	1	CHAPERONIN RING COMPLEX
E44	1	CHAPERONIN RINGS
E45	1	CHAPERONIN RNA-BINDING PROTEIN
E46	1	CHAPERONIN SEQUENCE DATABASE
E47	1	CHAPERONIN SUBSTRATE SPECTRUM
E48	4	CHAPERONIN SUBUNIT

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Ref	Items	Index-term
E49	2	CHAPERONIN SUBUNIT EPSILON
E50	3	CHAPERONIN SUBUNIT 5

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Ref	Items	RT	Index-term
E1	3		CHAPERONIN SUBUNIT 5

E2	1		CHAPERONIN SUBUNITS
E3	9		CHAPERONIN SYSTEM
E4	1		CHAPERONIN TRIC
E5	1		CHAPERONIN 1
E6	243	22	CHAPERONIN 10
E7	0	3	CHAPERONIN 10 (GROES)
E8	1		CHAPERONIN 10 --ADMINISTRATION AND DOSAGE --AD
E9	13		CHAPERONIN 10 --ANALYSIS --AN
E10	8		CHAPERONIN 10 --BIOSYNTHESIS --BI
E11	7		CHAPERONIN 10 --BLOOD --BL
E12	4		CHAPERONIN 10 --CHEMICAL SYNTHESIS --CS

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Set	Items	Description
S1	3067	E2-E23
S2	15326	E1-E50
S3	394244	'CHAPERONIN' OR DC='D4.680' OR R4:R7
S4	5	'CHAPERONIN COMPLEXES'
S5	4	'CHAPERONIN CPN60': 'CHAPERONIN CPN60 3'
S6	1	'CHAPERONIN GRO-EL OVERPRODUCTION' OR 'CHAPERONIN GRO-ES O- VERPRODUCTION'
S7	17	'CHAPERONIN PROTEIN': 'CHAPERONIN PROTEIN SUBUNITS'

? t s4/6/all

4/6/1 (Item 1 from file: 5)
0013395712 BIOSIS NO.: 200100567551
Assembly of chaperonin complexes
2001

4/6/2 (Item 2 from file: 5)
0012081326 BIOSIS NO.: 199900340986
On the role of symmetrical and asymmetrical chaperonin complexes in
assisted protein folding
1999

4/6/3 (Item 1 from file: 34)
12877081 Genuine Article#: 830AG Number of References: 35
Title: Cutaneous and sympathetic denervation in neonatal rats with a
mutation in the delta subunit of the cytosolic chap eronin-containing
t-complex peptide-1 gene (ABSTRACT AVAILABLE)
Publication date: 20040700

4/6/4 (Item 2 from file: 34)
10670689 Genuine Article#: BU33K Number of References: 91
Title: Persistent transmission of Luteoviruses by aphids
Publication date: 20020000

4/6/5 (Item 3 from file: 34)
06475487 Genuine Article#: YV845 Number of References: 48
Title: Anti-Ro/SS-A positivity and heat shock protein antibodies in
patients with normal-pressure glaucoma (ABSTRACT AVAILABLE)
Publication date: 19980200
? t s4/9/2 5

4/9/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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0012081326 BIOSIS NO.: 199900340986

On the role of symmetrical and asymmetrical chaperonin complexes in assisted protein folding

AUTHOR: Hayer-Hartl Manajit K (Reprint); Ewalt Karla L; Hartl F Ulrich
AUTHOR ADDRESS: Department of Cellular Biochemistry, Max-Planck-Institut fuer Biochemie, Am Klopferspitz 18A, D-82152, Martinsried, Germany**
Germany

JOURNAL: Biological Chemistry 380 (5): p531-540 May, 1999 1999

MEDIUM: print

ISSN: 1431-6730

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The cylindrical chaperonin GroEL of *E. coli* and its ring-shaped cofactor GroES cooperate in mediating the ATP-dependent folding of a wide range of polypeptides in vivo and in vitro. By binding to the ends of the GroEL cylinder, GroES displaces GroEL-bound polypeptide into an enclosed folding cage, thereby preventing protein aggregation during folding. The dynamic interaction of GroEL and GroES is regulated by the GroEL ATPase and involves the formation of asymmetrical GroEL:GroES1 and symmetrical GroEL:GroES2 complexes. The proposed role of the symmetrical complex as a catalytic intermediate of the chaperonin mechanism has been controversial. It has also been suggested that the formation of GroEL:GroES2 complexes allows the folding of two polypeptide molecules per GroEL reaction cycle, one in each ring of GroEL. By making use of a procedure to stabilize chaperonin complexes by rapid crosslinking for subsequent analysis by native PAGE, we have quantified the occurrence of GroEL:GroES1 and GroEL:GroES2 complexes in active refolding reactions under a variety of conditions using mitochondrial malate dehydrogenase (mMDH) as a substrate. Our results show that the symmetrical complexes are neither required for chaperonin function nor does their presence significantly increase the rate of mMDH refolding. In contrast, chaperonin-assisted folding is strictly dependent on the formation of asymmetrical GroEL:GroES1 complexes. These findings support the view that GroEL:GroES2 complexes have no essential role in the chaperonin mechanism.

DESCRIPTORS:

MAJOR CONCEPTS: Biochemistry and Molecular Biophysics

BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic

Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms

ORGANISMS: *Escherichia coli* (Enterobacteriaceae)

COMMON TAXONOMIC TERMS: Bacteria; Eubacteria; Microorganisms

CHEMICALS & BIOCHEMICALS: chaperonin complexes --assisted protein folding role, asymmetrical, symmetrical; mitochondrial malate dehydrogenase; protein--folding; GroEL--cylindrical chaperonin; GroEL:GroES-2 complex; GroES--ring-shaped cofactor

MISCELLANEOUS TERMS: chaperonin mechanism

CONCEPT CODES:

10060 Biochemistry studies - General

10802 Enzymes - General and comparative studies: coenzymes

BIOSYSTEMATIC CODES:

06702 Enterobacteriaceae

4/9/5 (Item 3 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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06475487 Genuine Article#: YV845 Number of References: 48

Title: Anti-Ro/SS-A positivity and heat shock protein antibodies in patients with normal-pressure glaucoma

Author(s): Wax MB (REPRINT) ; Tezel G; Saito I; Gupta RS; Harley JB; Li ZZ; Romano C

Corporate Source: WASHINGTON UNIV,SCH MED, DEPT OPHTHALMOL & VISUAL SCI, BOX 8096, 660 S EUCLID AVE/ST LOUIS//MO/63110 (REPRINT); WASHINGTON UNIV,SCH MED, DEPT ANAT & NEUROBIOL/ST LOUIS//MO/63110; SAGA MED SCH,DEPT OPHTHALMOL/SAGA//JAPAN/; MCMASTER UNIV,DEPT BIOCHEM/HAMILTON/ON/CANADA/; UNIV OKLAHOMA,HLTH SCI CTR, DEPT MED/OKLAHOMA CITY//OK/

Journal: AMERICAN JOURNAL OF OPHTHALMOLOGY, 1998, V125, N2 (FEB), P145-157

ISSN: 0002-9394 Publication date: 19980200

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Geographic Location: USA; JAPAN; CANADA

Subfile: CC LIFE--Current Contents, Life Sciences; CC CLIN--Current Contents, Clinical Medicine

Journal Subject Category: OPHTHALMOLOGY

Abstract: PURPOSE: To describe the clinical and laboratory findings in 10 patients with normal-pressure glaucoma and anti-Ro/SS-A positivity by enzyme linked immunosorbent assay (ELISA) and to determine whether that positivity may be related to an autoimmune mechanism for the optic neuropathy.

METHODS: In this prospective study, we evaluated ocular and systemic clinical findings of 10 patients with normal pressure glaucoma and anti-Ro/SS-A positivity by ELISA, including sicca complex features. Ouchterlony immunodiffusion was performed to confirm the presence of antibodies for Ro/SS-A, and the presence of other serum antibodies and their possible cross reactivities with Ro/SS-A were investigated.

RESULTS: None of the 10 patients with normal pressure glaucoma and anti-Ro/SS-A positivity (by ELISA) had clinical or laboratory signs of Sjogren syndrome or other connective tissue diseases. Only one of 10 patients had evidence of anti-Ro/SS-A antibodies by Ouchterlony immunodiffusion. All patients demonstrated serum immunoreactivity to bacterial heat shock protein 60 (hsp60) by Western blotting. Cross-reactivity between bacterial hsp60 and Ro/SS-A was demonstrated by Western blotting. Immunoreactivity to bacterial hsp60 by ELISA was significantly elevated in the sera of patients with normal-pressure glaucoma. Furthermore, patients with either normal-pressure or primary open-angle glaucoma had increased serum immunoreactivity to human hsp60.

CONCLUSIONS: Anti-Ro/SS-A positivity by ELISA in 10 patients with normal-pressure glaucoma was associated with a high level of serum immunoreactivity to bacterial hsp60, which may indicate that their glaucomatous optic neuropathy involves an as yet unidentified autoimmune mechanism. The identification of autoantibodies that react with human hsp60 in patients with normal pressure and primary open angle glaucoma may signify a common finding associated with the glaucomatous optic neuropathy process in some patients and appears to be unrelated to intraocular pressure levels.

Identifiers--Keyword Plus(R): RO SS-A; SYSTEMIC LUPUS-ERYTHEMATOSUS; PRIMARY SJOGRENS SYNDROME; DRY EYES; AUTOANTIGEN CALRETICULIN; CHAPERONIN COMPLEXES; CROSS-REACTIVITY; NORMAL SERA; AUTOANTIBODIES;

VASCULITIS

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Set	Items	Description
S1	3067	E2-E23
S2	15326	E1-E50
S3	394244	'CHAPERONIN' OR DC='D4.680' OR R4:R7
S4	5	'CHAPERONIN COMPLEXES'
S5	4	'CHAPERONIN CPN60': 'CHAPERONIN CPN60 3'
S6	1	'CHAPERONIN GRO-EL OVERPRODUCTION' OR 'CHAPERONIN GRO-ES O- VERPRODUCTION'

S7 17 'CHAPERONIN PROTEIN': 'CHAPERONIN PROTEIN SUBUNITS'
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3067 S1
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S8 410424 S1 OR S2 OR S3 OR S5 OR S6 OR S7
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410424 S8
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S9 472405 S8 OR CHAPERON?
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472405 S9
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4599 S10
44915 SONICAT?
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S11 0 S10 AND SONICAT? AND CENTRIFUG?
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Processing

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4599 S10
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? t s13/6/all

13/6/1 (Item 1 from file: 149)
01371830 SUPPLIER NUMBER: 12922676 (USE FORMAT 7 OR 9 FOR FULL TEXT)
Prevention of protein denaturation under heat stress by the chaperonin
Hsp60. (heat shock protein)

1992

WORD COUNT: 2763 LINE COUNT: 00272

? t s13/9/all

13/9/1 (Item 1 from file: 149)
DIALOG(R) File 149:TGG Health&Wellness DB(SM)
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01371830 SUPPLIER NUMBER: 12922676 (THIS IS THE FULL TEXT)
Prevention of protein denaturation under heat stress by the chaperonin
Hsp60. (heat shock protein)

Martin, Jorg; Horwich, Arthur L.; Hartl, F. Ulrich
Science, v258, n5084, p995(4)
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1992

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TEXT:

The increased synthesis of heat shock proteins is a ubiquitous physiological response of cells to environmental stress. How these proteins function in protecting cellular structures is not yet understood. The mitochondrial heat shock protein 60 (Hsp60) has now been shown to form complexes with a variety of polypeptides in organelles exposed to heat stress. The Hsp60 was required to prevent the thermal inactivation in vivo of native dihydrofolate reductase (DHFR) imported into mitochondria. In vitro, Hsp60 bound to DHFR in the course of thermal denaturation, preventing its aggregation, and mediated its adenosine triphosphate-dependent refolding at increased temperatures. These results suggest a general mechanism by which heat shock proteins of the Hsp60 family stabilize preexisting proteins under stress conditions.

On exposure to various forms of environmental stress, cells generally respond by increasing the rate of synthesis of a set of highly conserved stress, or heat shock, proteins (Hsps) [1]. Many stress proteins, including members of the Hsp70 and Hsp60 families, are constitutively expressed and fulfill essential functions as "molecular chaperones" [2] under normal cellular conditions. The Hsp60s, which are found in bacterial cytosol as well as in mitochondria and chloroplasts, are high molecular mass, double-ring complexes consisting of 14 [unkeyable]60-kD subunits [2]. These "chaperonins" [3] interact with early intermediates in the protein folding pathway and mediate the acquisition of the native structure of newly synthesized proteins by releasing the substrate in an adenosine triphosphate (ATP)-dependent process [4-8]. The ATP-hydrolytic activity of chaperonins is regulated by smaller co- chaperonins , ring complexes of seven [unkeyable]10-kD subunits [4, 9].

Although most conditions that induce the stress response potentially lead to the accumulation of denatured proteins [10], little is known about the mechanisms by which Hsps might function in preventing denaturation or in renaturing damaged proteins [1]. On exposure of *Escherichia coli* to temperatures between 42[degrees] and 46[degrees]C, the concentration of the bacterial chaperonin GroEL increases five- to tenfold, reaching up to 12% of total cellular protein. Similarly, the concentration of the mitochondrial Hsp60 of yeast increases two- to threefold at 42[degrees]C [8, 11], and that of the co-chaperonin Hsp10 of mammalian mitochondria approximately tenfold under these conditions [12]. Such induction may reflect an increased requirement for these Hsps in protecting preexisting proteins from denaturation.

A large number of polypeptides were bound to mitochondrial Hsp60 when isolated mitochondria of [³⁵S]-labeled *Neurospora crassa* were incubated for 10 min at increased temperatures (Fig. 1). Mitochondrial extracts [13] were analyzed by electrophoresis on native polyacrylamide gels, on which Hsp60-bound polypeptides comigrate with the Hsp60 complex. When the bands corresponding to Hsp60 were excised and subjected to electrophoresis on reducing SDS-polyacrylamide gels, a range of polypeptides of 10 to 90 kD that had been associated with Hsp60 was apparent. The amount of bound protein increased significantly after shifting the temperature from 32[degrees] to 39[degrees] or 46[degrees]C (Fig. 1). A small number of proteins comigrated with Hsp60 already at 25[degrees]C, an unidentified polypeptide of [unkeyable]34 kD being most prominent. The total amount of [³⁵S] present in the Hsp60-associated polypeptides corresponded to [unkeyable]10% of that in Hsp60, which is consistent with the observed capacity of the 14-subunit chaperonin to bind only one to two substrate molecules [4-6, 14]. The Hsp60-associated proteins did not represent degradation products of Hsp60 [15]. Protein binding to Hsp60 occurred in the intact mitochondria because it was not prevented when a large excess of [unkeyable.sub.s1]-casein was present during preparation of the extracts (Fig. 1); casein binds to the chaperonin despite its stability in solution [4]. Similar results were obtained with *Saccharomyces cerevisiae*

mitochondria [16].

Apparently, Hsp60 recognizes a variety of preexisting mitochondrial proteins as they become denatured under heat stress. These proteins were found associated with Hsp60 despite the presence of ATP in mitochondria, perhaps cycling between free and Hsp60-bound states at the increased temperature. Consistent with this proposal, the amount of bound protein decreased considerably when the temperature was lowered from 39[degrees] to 25[degrees]C [16]; it was unclear, however, whether this protein was released from Hsp60 or whether it was proteolytically degraded. To address this question, we analyzed the interaction of purified chaperonin with [sup.35S]-labeled proteins in extracts of yeast mitochondria and cytosol. GroEL, the *E. coli* homolog of Hsp60, was used in these experiments because both GroEL and its co-chaperonin GroES are easily purified from an overproducing strain of *E. coli*, whereas the mitochondrial GroES is rather difficult to isolate [9, 12]. Furthermore, GroEL could be distinguished on native gels from the [sup.35S]-labeled Hsp60 in mitochondrial extracts. Again, a wide range of polypeptides bound to GroEL in a temperature-dependent manner, but only when the chaperonin was present during incubation at the increased temperature (Fig. 2A). When GroEL was added to the extracts after a shift from 50[degrees] to 25[degrees]C, the amount of bound protein was reduced to the level seen before heat treatment. Proteins bound to GroEL at 40[degrees]C were at least partially released on incubation with MgATP at 25[degrees]C (Fig. 2B). ATP-dependent release was enhanced by the addition of GroES, more efficiently with cytosolic than with mitochondrial proteins [13]. The discharged proteins were soluble, which suggests that they were in their native conformations [17].

Dihydrofolate reductase (DHFR) was chosen as a relatively thermolabile model protein [Gibbs free energy of unfolding ([unkeyable]G.sup.0)[sub.unfolding] = 5.9 kcal/mol [18]] to test whether Hsp60 was able to preserve the functional integrity of proteins during heat shock. DHFR, normally a cytosolic protein, interacts with Hsp60 when it is directed into mitochondria by a targeting sequence fused to the NH[sub.2]-terminus [7]. A fusion protein, pOTC-DHFR, containing the cleavable presequence of human ornithine transcarbamoylase (OTC) [19], was expressed from a galactose-regulated promoter in wild-type and in *mif4* yeast. In the *mif4* mutant, Hsp60 loses its function at the nonpermissive temperature of 37[degrees]C and becomes insoluble [8]. pOTC-DHFR was efficiently imported into mitochondria and was processed to the mature form (mOTC-DHFR), which contains nine linker residues NH[sub.2]-terminal to the DHFR sequence (Fig. 3) [19]. mOTC-DHFR constituted 0.5 to 1% of the total mitochondrial matrix protein and possessed [unkeyable]85% of the specific enzymatic activity of authentic DHFR [16]. When expressed at the permissive temperature (23[degrees]C), DHFR could be extracted from the isolated mitochondria of wild-type and *mif4* cells in a soluble, enzymatically active form (Fig. 3A). In mitochondria of mutant cells grown at 37[degrees]C, most of the DHFR protein was inactive and was recovered in the insoluble pellet fraction, whereas in wild-type organelles, DHFR remained soluble and active at 37[degrees]C.

The aggregated DHFR in *mif4* cells could have been derived from preexisting protein that denatured at 37[degrees]C or from newly imported protein that was unable to fold. The following experiments were performed to address these possibilities. In the first experiment, pOTC-DHFR synthesis was induced by galactose at 23[degrees]C, and then expression was repressed by adding glucose [20]. Subsequently, the culture was shifted to 37[degrees]C to induce expression of the *mif4* phenotype. Mostly insoluble mOTC-DHFR was detected in the mitochondria (Fig. 3A). Compared to *mif4* cells that were maintained at 23[degrees]C, the amount of DHFR protein in an equivalent amount of mitochondrial protein was reduced by [unkeyable]30 to 50%, probably because of degradation. The specific activity of mOTC-DHFR in *mif4* mitochondria at 37[degrees]C was 25% of that in the wild-type

control. Apparently, some aggregated DHFR renatured upon dilution in the presence of substrates. In the second experiment, pOTC-DHFR was expressed after the cells were shifted to the nonpermissive temperature. Both precursor and mature protein were then present in the mitochondria in an insoluble form (Fig. 3A). Under these conditions, DHFR-specific activity was approximately one-third of the wild-type activity when equal amounts of mature-size protein were compared. The accumulation of uncleaved precursor in mif4 mitochondria suggested that the newly imported proteins adopted misfolded conformations that were not readily accessible to proteolytic processing. Again, there was considerably less total DHFR in mif4 mitochondria under these conditions than in wild-type organelles. Assuming similar levels of expression, either the mutant mitochondria had a reduced efficiency of protein import at 37[degrees]C or, more likely, misfolded protein was degraded [21]. This degradation may explain why mif4 mitochondria do not accumulate large amounts of protein aggregates at 37[degrees]C when cell growth ceases [8]. In summary, these results indicate a requirement for functional Hsp60 for maintaining preexisting, folded DHFR in an active conformation at increased temperatures, in addition to Hsp60's function in folding the newly imported protein [7] (Fig. 3A).

Several authentic proteins of the mitochondrial matrix, although more stable than DHFR, were similarly affected in mif4 cells at 37[degrees]C. Without specifically distinguishing between protein that had been imported before and after the shift to 37[degrees]C, we recovered [unkeyable]20 to 30% of total malate dehydrogenase (MDH) and citrate synthase in Genapol-insoluble forms after 1 hour at the nonpermissive temperature [16].

The function of Hsp60 in protein folding generally requires ATP hydrolysis [4-7]. Thus, it seemed likely that maintenance of DHFR in an active state would also be dependent on ATP. In isolated, energized wild-type mitochondria, 80% of DHFR remained soluble and active after incubation for 15 min at 40[degrees]C (Fig. 3B). In contrast, only 30% of the original DHFR activity was measured in ATP-depleted mitochondria; [unkeyable]70% of total DHFR had aggregated. Aggregated DHFR was not bound to Hsp60, which remained soluble. A significant amount of the soluble DHFR was present in a high molecular mass complex with Hsp60 (Fig. 3C) and could be co-immunoprecipitated with antibodies to Hsp60 [16]. No interaction with Hsp60 was observed at 25[degrees]C. The effects of the 15-min heat treatment on authentic mitochondrial proteins were variable; for example, 30% of citrate synthase and 15% of MDH formed insoluble aggregates in an ATP-dependent manner [16]. DHFR remained soluble at 40[degrees]C in ATP-depleted mitochondria that were incubated in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and dihydrofolate [16, 22]. Substrate binding is known to stabilize the native conformation of DHFR [18]. Thus, small differences in the stability of the native state appear to determine the fate of a protein at the upper end of the physiologically relevant temperature scale.

We reproduced in vitro the chaperonin function in stabilizing DHFR under heat stress with purified GroEL and GroES proteins. The effects of GroEL were first analyzed in the absence of ATP, which allowed protein binding but not release. Native DHFR was incubated with a twofold molar excess of GroEL for 5 min at temperatures between 25[degrees] and 70[degrees]C, and enzyme activities were determined after cooling to 25[degrees]C (Fig. 4A). The thermal deactivation curve of DHFR was lowered by [unkeyable]15[degrees]C in the presence of GroEL. Apparently, GroEL bound to thermally destabilized DHFR, thus shifting the equilibrium from the folded to the unfolded state. In contrast, a recent study demonstrating the binding of GroEL to thermally denatured [unkeyable]-glucosidase in vitro showed no influence of GroEL on the kinetics of denaturation [23]. The thermal denaturation of DHFR observed in the absence of the chaperonin was irreversible [18]; subsequent addition of GroEL and MgATP (with or without GroES) did not result in reactivation [16]. However, DHFR that had

been incubated with GroEL at temperatures up to 65[degrees]C (GroEL itself denatured between 65[degrees] and 70[degrees]C) could be reactivated by incubation with MgATP at 25[degrees]C (Fig. 4A). Omission of MgATP during the second incubation at 25[degrees]C resulted in further inactivation of DHFR because of GroEL binding [6]. DHFR that had associated with GroEL at 40[degrees]C showed intrinsic tryptophan fluorescence properties very similar to those described for the GroEL-associated protein bound after dilution from denaturant (Fig. 4B) [4]. Both of the chaperonin-associated forms of DHFR exhibited an equally high sensitivity toward protease [16].

Finally, we investigated whether the chaperonin system was able to maintain DHFR in an enzymatically active state at 40[degrees]C, simulating the heat shock conditions applied in organello. Free DHFR lost its activity with a half-time of [unkeyable]10 min (Fig. 4C), whereas in the presence of GroEL the rate of deactivation was increased. Upon incubation with GroEL and MgATP, DHFR was only slightly more stable than in the absence of the chaperonin. GroEL, MgATP, and GroES were required to efficiently preserve the activity of DHFR at 40[degrees]C, thus reproducing the chaperonin function observed in intact mitochondria. Even substoichiometric concentrations of GroEL versus DHFR had a significant stabilizing effect. In contrast, equivalent molar concentrations of bovine serum albumin did not stabilize DHFR at 40[degrees]C [16].

Partial folding of DHFR can occur in close association with GroEL or Hsp60 in an environment shielded from other unfolded polypeptides [4, 7]. This mechanism may also underlie the chaperonin function in preserving the active state of proteins under heat stress. GroEL and Hsp60 appear to bind to (partially) unfolded protein molecules, which are relatively more abundant at increased temperatures, thus preventing their (irreversible) aggregation. A folding step before release would reduce the concentration of these unfolded species. If this reaction does not result in productive folding--for example, at high temperature--the proteins may rebind to GroEL or Hsp60. They might renature in a chaperonin-dependent reaction after more favorable conditions are established, or they might be channeled into degradative pathways [24]. Such a function of the chaperonin system would explain its required presence during thermal denaturation. Some proteins may rely on an interaction with GroEL or Hsp60 even under nonstressful conditions, undergoing repeated unfolding and refolding. The chaperonins appear to be without effect after protein aggregates have formed [4, 6]. In contrast, the Hsp70 homolog DnaK has the capacity to renature proteins when added after thermal inactivation [25]. Clearly, the Hsp70 in the mitochondrial matrix, the function of which is required for protein import and subsequent folding [26], was not sufficient to maintain the folded state of DHFR at 37[degrees]C or to mediate its de novo formation in the mif4 mutant. Notably, the mitochondrial Hsp70 remains functional in the mif4 strain [8, 26]. Nevertheless, it seems possible that the Hsp70 and Hsp60 systems cooperate in preventing protein denaturation under stress conditions. A sequential pathway of Hsp70 and Hsp60 action in de novo protein folding has already been described [27].

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DESCRIPTORS: Proteins--Demonstrations, protests, etc.; Heat shock proteins

--Physiological aspects; Stress (Physiology)--Research

FILE SEGMENT: MI File 47

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00501309 (USE 9 FOR FULLTEXT)

Prevention of Mucosal Escherichia coli Infection by FimH-Adhesin-Based Systemic Vaccination

Langermann, Solomon; Palaszynski, Susan; Barnhart, Michelle; Auguste, Gale; Pinkner, Jerome S.; Burlein, Jeanne; Barren, Philip; Koenig, Scott; Leath, Simon; Jones, C. Hal; Hultgren, Scott J.

S. Langermann, S. Palaszynski, G. Auguste, J. Burlein, P. Barren, S. Koenig, S. Leath, MedImmune, Inc., Gaithersburg, MD 20878, USA. ; M. Barnhart, J. S. Pinkner, C. Hal Jones, S. J. Hultgren, Department of Molecular Microbiology, Washington University, St. Louis, MO 63110, USA. Science Vol. 276 5312 pp. 607

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...Text: to type 1-piliated E. coli. Antibody was raised in mice to two forms of purified FimH protein: (i) a complex containing the periplasmic chaperone FimC bound to the full-length FimH protein (FimC-H), and (ii) a naturally occurring...

...FimHt), corresponding to the NH₂-terminal two-thirds of the FimH protein, was purified away from the FimC-H complex (B8). Antibody was also raised to whole type 1...immunoglobulin G (IgG) titers to (A) FimHt adhesin and (B) whole type 1 pili after immunization with purified adhesin, adhesin-chaperone complex, or whole type 1 pili. The serum immune responses to FimH and whole type 1 pili were evaluated after primary immunization (day 0) [in complete Freund's adjuvant (CFA)] and booster immunization (week 4) [in incomplete Freund's adjuvant (IFA)] with purified FimHt (squares), FimC-H (circles), or whole type 1 pili (triangles) (B8). The immunogenicity of...

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(54) Title: STRESS PROTEIN-PEPTIDE COMPLEXES AS PROPHYLACTIC AND THERAPEUTIC VACCINES AGAINST INTRACELLULAR PATHOGENS			
(57) Abstract Disclosed is a family of vaccines that contain stress protein-peptide complexes which when administered to a mammal are operative to initiate in the mammal a cytotoxic T cell response against cells infected with a preselected intracellular pathogen. Also disclosed are methodologies for preparing and administering vaccines containing such stress protein-peptide complexes.			

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TITLE: STRESS PROTEIN-PEPTIDE COMPLEXES AS PROPHYLACTIC AND THERAPEUTIC VACCINES AGAINST INTRACELLULAR PATHOGENS

Abstract Text (1):

CHG DATE=19990617 STATUS=O>Disclosed is a family of vaccines that contain stress protein-peptide complexes which when administered to a mammal are operative to initiate in the mammal a cytotoxic T cell response against cells infected with a preselected intracellular pathogen. Also disclosed are methodologies for preparing and administering vaccines containing such stress protein-peptide complexes.

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Mar 11, 2004

DOCUMENT-IDENTIFIER: US 20040048353 A1

TITLE: *Helicobacter pylori* proteins useful for vaccines and diagnostics

CLAIMS:

1. A recombinant *Helicobacter pylori* protein, or a derivative or fragment thereof.
2. The recombinant protein according to claim 1 wherein the protein is a *Helicobacter pylori* cytotoxin or a precursor, derivative or fragment thereof.
4. The recombinant protein according to claim 1 wherein the protein is a *Helicobacter pylori* cytotoxin associated immunodominant antigen, or a derivative or fragment thereof.
6. The recombinant protein according to claim 1 wherein the protein is a *Helicobacter pylori* heat shock protein, or a derivative or fragment thereof.
13. The recombinant protein according to any one of claims 1 to 11 for use in the treatment of *Helicobacter pylori* infection.
17. The vaccine or therapeutic composition according to claim 16 comprising, in combination, two or more of i) a recombinant *Helicobacter pylori* cytotoxic protein precursor, derivative or fragment thereof, ii) a *Helicobacter pylori* recombinant cytotoxin associated immunodominant antigen, or a derivative or fragment thereof, iii) *Helicobacter pylori* recombinant heat shock protein or a derivative or fragment thereof and/or iv) a *Helicobacter pylori* urease.
22. Use of one or more recombinant proteins according to any one of claims 1 to 11 for the manufacture of a medicament for the treatment of *Helicobacter pylori* infection.
23. A method of treatment of an individual infected with *Helicobacter pylori* comprising administering an effective amount of a recombinant protein according to 1 to 11.
24. The method of treatment according to claim 23 comprising administering an effective amount of, in combination, two or more of i) a recombinant *Helicobacter pylori* cytotoxic protein precursor, derivative or fragment thereof, ii) a *Helicobacter pylori* recombinant cytotoxin associated immunodominant antigen, or a derivative or fragment thereof, iii) a *Helicobacter pylori* recombinant heat shock protein or a derivative or fragment thereof and/or iv) a *Helicobacter pylori* urease.
25. A method of vaccination comprising administering an immunologically effective amount of, in combination, two or more of i) a recombinant *Helicobacter pylori* cytotoxic protein precursor, derivative or fragment thereof, ii) a *Helicobacter pylori* recombinant cytotoxin associated immunodominant antigen, or a derivative or fragment thereof, iii) a *Helicobacter pylori* recombinant heat shock protein or a derivative or fragment thereof and/or iv) a *Helicobacter pylori* urease.

27. A recombinant polynucleotide encoding a Helicobacter pylori cytotoxic protein or a derivative or fragment thereof comprising all or part of the nucleotide sequence of FIG. 1.

28. A recombinant polynucleotide encoding a Helicobacter pylori recombinant cytotoxin associated immunodominant antigen or a derivative or fragment thereof comprising all or a part of the nucleotide sequence of FIG. 4.

29. A recombinant polynucleotide encoding a Helicobacter pylori recombinant heat shock protein or a derivative or fragment thereof comprising all or a part of the nucleotide sequence of FIG. 5.

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(12) **United States Patent**
Weltzin et al.

(10) Patent No.: **US 6,576,244 B1**
(45) Date of Patent: **Jun. 10, 2003**

(54) **LT AND CT IN PARENTERAL
IMMUNIZATION METHODS AGAINST
HELICOBACTER INFECTION**

(75) Inventors: **Richard A. Weltzin, Lunenburg, MA
(US); Bruno Guy, Lyons (FR)**

(73) Assignee: **Acambis, Inc., Cambridge, MA (US)**

(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/336,115**

(22) Filed: **Jun. 18, 1999**

Related U.S. Application Data

(63) Continuation-in-part of application No. 09/100,258, filed on
Jun. 19, 1998.

(51) Int. Cl.⁷ **A61K 39/02**

(52) U.S. Cl. **424/234.1; 424/236.1;
424/184.1; 424/94.6; 514/12; 530/350;
530/403**

(58) Field of Search **424/234.1, 236.1,
424/184.1, 94.6; 435/7.1, 252.8; 530/350,
403; 514/12**

(56) **References Cited**

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Assistant Examiner—Ginny Allen Portner

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(57) **ABSTRACT**

This invention provides methods of inducing a protective or therapeutic immune response to Helicobacter infection in a mammal by parenterally administering to the mammal one or more Helicobacter antigens and an adjuvant selected from one or more of LT, CT, LTB, and CTB.

14 Claims, 11 Drawing Sheets

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L7: Entry 1 of 3

File: USPT

Jun 10, 2003

US-PAT-NO: 6576244

DOCUMENT-IDENTIFIER: US 6576244 B1

TITLE: LT and CT in parenteral immunization methods against helicobacter infection

DATE-ISSUED: June 10, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Weltzin; Richard A.	Lunenburg	MA		
Guy; Bruno	Lyons			FR

US-CL-CURRENT: 424/234.1; 424/184.1, 424/236.1, 424/94.6, 514/12, 530/350, 530/403

CLAIMS:

What is claimed is:

1. A method of inducing an immune response to Helicobacter in a mammal, said method comprising administering to said mammal by injection (a) an immunogenic Helicobacter pylori polypeptide that is admixed with (b) an adjuvant comprising immunogenic Helicobacter pylori polypeptide that is admixed with (b) an adjuvant comprising one or more of (i) heat-labile toxin of *Escherichia coli*, (ii) the B subunit of the heat-labile toxin of *Escherichia coli*, (iii) cholera toxin, and (iv) the B subunit of cholera toxin.
2. The method of claim 1, wherein the polypeptide and the adjuvant are provided together in a solution.
3. The method of claim 1, wherein the polypeptide comprises Helicobacter pylori urease or a subunit or immunogenic fragment thereof.
4. The method of claim 1, wherein the heat-labile toxin of *Escherichia coli* and the B subunit of the heat-labile toxin of *Escherichia coli* are administered to said mammal.
5. The method of claim 1, wherein said injection is subcutaneous.
6. The method of claim 1, wherein said injection is intradermal.
7. The method of claim 1, wherein said Helicobacter pylori polypeptide comprises catalase or an immunogenic fragment thereof.
8. The method of claim 1, wherein said Helicobacter pylori polypeptide comprises a polypeptide selected from the group consisting of HspA, HspB, lactoferrin receptor, p76 (SEQ ID NOs:1-22), p32 (SEQ ID NOs:23 and 24), Baba,

BabB, AlpA, AlpB, and immunogenic fragments thereof.

9. The method of claim 1, further comprising administering to said mammal one or more additional immunogenic Helicobacter pylori polypeptides.

10. The method of claim 9, wherein said Helicobacter pylori polypeptide is urease and said one or more additional Helicobacter pylori polypeptides is selected from the group consisting of catalase, HspA, HspB, lactoferrin receptor, p76 (SEQ ID NOs:1-22), p32 (SEQ ID NOs:23 and 24), BabA, BabB, AlpA, AlpB, and immunogenic fragments thereof.

11. The method of claim 1, wherein said Helicobacter pylori polypeptide comprises a subunit of Helicobacter pylori urease.

12. The method of claim 1, wherein said Helicobacter pylori polypeptide comprises Helicobacter pylori catalase.

13. The method of claim 1, wherein said Helicobacter pylori polypeptide comprises a Helicobacter pylori polypeptide selected from the group consisting of catalase, HspA, HspB, lactoferrin receptor, p76 (SEQ ID NOs:1-22), p32 (SEQ ID NOs:23 and 24), BabA, BabB, AlpA, and AlpB.

14. A method of inducing a protective or therapeutic immune response to Helicobacter infection in a mammal, said method comprising administering to said mammal by injection (a) a polypeptide comprising a subunit of Helicobacter pylori urease that is admixed with (b) an adjuvant comprising one or more of (i) heat-labile toxin of Escherichia coli, (ii) the B subunit of the heat-labile toxin of Escherichia coli, (iii) cholera toxin, and (iv) the B subunit of cholera toxin.

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Glu Val Lys Lys Val Gln Ile Pro Glu Lys Glu Met Gln Asp Phe Tyr	
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125	130 135 140
His Ile Leu Val Lys Thr Glu Asp Glu Ala Lys Arg Ile Ile Ser Glu	
	145 150 155
Ile Asp Lys Gln Pro Lys Ala Lys Lys Glu Ala Lys Phe Ile Glu Leu	
	160 165 170
Ala Asn Arg Asp Thr Ile Asp Pro Asn Ser Lys Asn Ala Gln Asn Gly	
	175 180 185
Gly Asp Leu Gly Lys Phe Gln Lys Asn Gln Met Ala Pro Asp Phe Ser	
	190 195 200
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Pro Val Thr Tyr Thr Tyr Glu Gln Ala Lys Pro Thr Ile Lys Gly Met	
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Leu Gln Glu Lys Leu Phe Gln Glu Arg Met Asn Gln Arg Ile Glu Glu	
	255 260 265
Leu Arg Lys His Ala Lys Ile Val Ile Asn Lys	
270	275

What is claimed is:

1. A method of inducing an immune response to *Helicobacter* in a mammal, said method comprising administering to said mammal by injection (a) an immunogenic *Helicobacter pylori* polypeptide that is admixed with (b) an adjuvant comprising immunogenic *Helicobacter pylori* polypeptide that is admixed with (b) an adjuvant comprising one or more of (i) heat-labile toxin of *Escherichia coli*, (ii) the B subunit of the heat-labile toxin of *Escherichia coli*, (iii) cholera toxin, and (iv) the B subunit of cholera toxin.

2. The method of claim 1, wherein the polypeptide and the adjuvant are provided together in a solution.

3. The method of claim 1, wherein the polypeptide comprises *Helicobacter pylori* urease or a subunit or immunogenic fragment thereof.

4. The method of claim 1, wherein the heat-labile toxin of *Escherichia coli* and the B subunit of the heat-labile toxin of *Escherichia coli* are administered to said mammal.

5. The method of claim 1, wherein said injection is subcutaneous.

6. The method of claim 1, wherein said injection is intradermal.

7. The method of claim 1, wherein said *Helicobacter pylori* polypeptide comprises catalase or an immunogenic fragment thereof.

8. The method of claim 1, wherein said *Helicobacter pylori* polypeptide comprises a polypeptide selected from the group consisting of HspA, HspB, lactoferrin receptor, p76 (SEQ ID NOs:1-22), p32 (SEQ ID NOs:23 and 24), BabA, BabB, AlpA, AlpB, and immunogenic fragments thereof.

9. The method of claim 1, further comprising administering to said mammal one or more additional immunogenic *Helicobacter pylori* polypeptides.

10. The method of claim 9, wherein said *Helicobacter pylori* polypeptide is urease and said one or more additional *Helicobacter pylori* polypeptides is selected from the group consisting of catalase, HspA, HspB, lactoferrin receptor, p76 (SEQ ID NOs:1-22), p32 (SEQ ID NOs:23 and 24), BabA, BabB, AlpA, AlpB, and immunogenic fragments thereof.

11. The method of claim 1, wherein said *Helicobacter pylori* polypeptide comprises a subunit of *Helicobacter pylori* urease.

12. The method of claim 1, wherein said *Helicobacter pylori* polypeptide comprises *Helicobacter pylori* catalase.

13. The method of claim 1, wherein said *Helicobacter pylori* polypeptide comprises a *Helicobacter pylori* polypeptide selected from the group consisting of catalase, HspA, HspB, lactoferrin receptor, p76 (SEQ ID NOs:1-22), p32 (SEQ ID NOs:23 and 24), BabA, BabB, AlpA, and AlpB.

14. A method of inducing a protective or therapeutic immune response to *Helicobacter* infection in a mammal, said method comprising administering to said mammal by injection (a) a polypeptide comprising a subunit of *Helicobacter pylori* urease that is admixed with (b) an adjuvant comprising one or more of (i) heat-labile toxin of *Escherichia coli*, (ii) the B subunit of the heat-labile toxin of *Escherichia coli*, (iii) cholera toxin, and (iv) the B subunit of cholera toxin.

* * * * *



US005843460A

United States Patent [19]

Labigne et al.

[11] Patent Number: **5,843,460**[45] Date of Patent: **Dec. 1, 1998**

[54] **IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS, AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES**

[75] Inventors: Agnes Labigne, Bures S/Yvette, France; Sebastin Suerbaum, Bochum, Germany; Richard L. Ferrero, Paris; Jean-Michel Thiberge, Plaisir, both of France

[73] Assignees: Institut Pasteur; Institut National de la Sante et de la Recherche Medicale, both of Paris, France

[21] Appl. No.: 467,822

[22] Filed: Jun. 6, 1995

Related U.S. Application Data

[63] Continuation of Ser. No. 447,177, May 19, 1995, which is a continuation-in-part of Ser. No. 432,697, May 2, 1995.

Foreign Application Priority Data

May 19, 1993 [EP] European Pat. Off. 93 401 309
Nov. 19, 1993 [WO] WIPO PCT/EP93/03259

[51] Int. Cl.⁶ A61K 39/02

[52] U.S. Cl. 424/234.1; 435/7.32; 435/6; 435/7.9; 514/234.5; 514/41

[58] Field of Search 435/7.32, 4, 6, 435/7.9; 514/234.5, 41; 424/234.1

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(List continued on next page.)

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Assistant Examiner—Ginny Allen Portner

Attorney, Agent, or Firm—Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P.

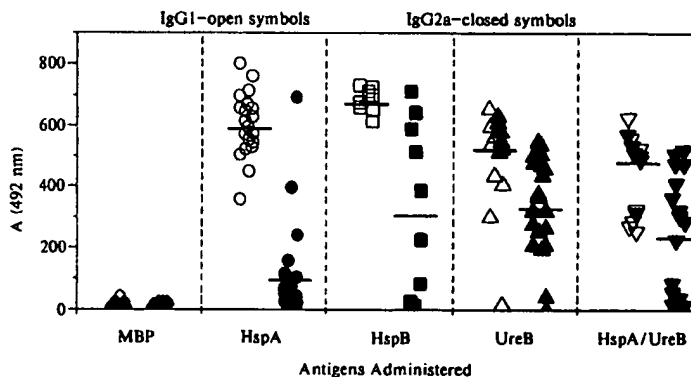
[57] ABSTRACT

There is provided an immunogenic composition capable of inducing protective antibodies against *Helicobacter infection* characterized in that it comprises:

- i) at least one sub-unit of a urease structural polypeptide from *Helicobacter pylori* (SEQ ID NOS:22,26), or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter felis* urease (SEQ ID NOS:20-21), and/or at least one sub-unit of a urease structural polypeptide from *Helicobacter felis* (SEQ ID NOS:20-21), or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter pylori* urease (SEQ ID NOS:22-26);
- ii) and/or, a heat shock protein (Hsp), or chaperonin, from *Helicobacter*, or a fragment of said protein.

The preparation, by recombinant means, of such immunogenic compositions is also provided.

10 Claims, 36 Drawing Sheets



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L7: Entry 3 of 3

File: USPT

Dec 1, 1998

US-PAT-NO: 5843460

DOCUMENT-IDENTIFIER: US 5843460 A

TITLE: Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides

DATE-ISSUED: December 1, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Labigne; Agnes	Bures S/Yvette			FR
Suerbaum; Sebastin	Bochum			DE
Ferrero; Richard L.	Paris			FR
Thiberge; Jean-Michel	Plaisir			FR

US-CL-CURRENT: 424/234.1; 435/6, 435/7.32, 435/7.9, 514/234.5, 514/41

CLAIMS:

We claim:

1. An immunogenic composition, capable of inducing antibodies against Helicobacter infection, comprising:

i) at least one urease structural polypeptide encoded by the UreB gene of Helicobacter pylori or Helicobacter felis or immunogenic fragment thereof comprising at least six consecutive amino acids; and

ii) at least one heat shock protein encoded by the Hsp A gene of Helicobacter pylori or Helicobacter felis or immunogenic fragment thereof, comprising at least 6 consecutive amino acids,

said composition being substantially free of other Helicobacter pylori or Helicobacter felis proteins.

2. An immunogenic composition comprising an immunizing amount of a mixture of Helicobacter pylori or Helicobacter felis antigens, wherein said mixture consists essentially of UreB and HspA of H. pylori or H. felis substantially free of other H. pylori or H felis proteins.

3. The immunogenic composition according to claim 1 or claim 2, wherein the HspA is encoded by the HspA gene of plasmid pILL689 (CNCM I-1356).

4. The immunogenic composition according to claim 1 or claim 2, wherein the HspA comprises the amino acid sequence of SEQ ID NO: 1 or an immunogenic fragment thereof having at least 6 consecutive amino acids.

5. The immunogenic composition according to claim 1 or claim 2, additionally comprising an adjuvant.
6. The immunogenic composition according to claim 1 or claim 2, wherein said composition produces an immunogenic effect when administered to a mammal, wherein the immunogenic effect is substantially the same as the immunogenic effect produced in the mammal when a total cell extract of Helicobacter pylori or Helicobacter felis is administered to said mammal.
7. A method of inducing an immune response in an animal, comprising the step of administering to the animal an immunizing amount of a composition according to claim 1 or claim 2.
8. The method according to claim 7, wherein the animal is a cat or a dog.
9. The method according to claim 7, wherein the animal is a human.
10. A pharmaceutical composition for use in a vaccine against Helicobacter pylori or Helicobacter felis, comprising the immunogenic composition according to claim 1 or claim 2, in combination with a pharmaceutically acceptable carrier.

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US006248330B1

(12) **United States Patent**
Labigne et al.

(10) Patent No.: **US 6,248,330 B1**
(45) Date of Patent: ***Jun. 19, 2001**

(54) **IMMUNOGENIC COMPOSITIONS AGAINST
HELICOBACTER INFECTION,
POLYPEPTIDES FOR USE IN THE
COMPOSITIONS, AND NUCLEIC ACID
SEQUENCES ENCODING SAID
POLYPEPTIDES**

(75) Inventors: Agnes Labigne, Bures sur Yvette (FR);
Sebastien Suerbaum, Bochum (DE);
Richard L. Ferrero, Paris;
Jean-Michel Thiberge, Plaisir, both of
(FR)

(73) Assignee: Institut Pasteur, Paris (FR)

(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.

This patent is subject to a terminal dis-
claimer.

(21) Appl. No.: 08/432,697

(22) Filed: May 2, 1995

Related U.S. Application Data

(63) Continuation-in-part of application No. PCT/EP94/01625,
filed on May 19, 1994.

(30) Foreign Application Priority Data

May 19, 1993 (EP) 93401309
May 19, 1994 (WO) PCT/EP94/03259

(51) Int. Cl.⁷ A61K 39/00

(52) U.S. Cl. 424/192.1; 424/234.1;
424/184.1; 435/6; 435/69.1

(58) Field of Search 424/234.1, 184.1,
424/203.1, 192.1; 435/6, 7.21

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WOA9109049 6/1991 (WO).
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English).*

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Assistant Examiner—Ginny Allen Portner

(74) Attorney, Agent, or Firm—Finnegan, Henderson,
Farabow, Garrett & Dunner

(57)

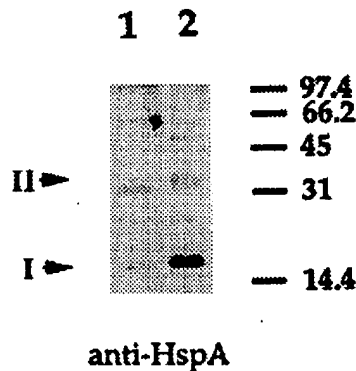
ABSTRACT

There is provided an immunogenic composition capable of
inducing protective antibodies against *Helicobacter* infec-
tion characterized in that it comprises:

- i) at least one sub-unit of a urease structural polypeptide
from *Helicobacter pylori*, or a fragment thereof, said
fragment being recognized by antibodies reacting with
Helicobacter felis urease, and/or at least one sub-unit of
a urease structural polypeptide from *Helicobacter felis*,
or a fragment thereof, said fragment being recognized
by antibodies reacting with *Helicobacter pylori* urease;
- ii) and/or, a heat shock protein (Hsp), or chaperonin, from
Helicobacter, or a fragment of said protein.

The preparation, by recombinant means, of such immuno-
genic compositions is also provided.

16 Claims, 36 Drawing Sheets



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L7: Entry 2 of 3

File: USPT

Jun 19, 2001

US-PAT-NO: 6248330

DOCUMENT-IDENTIFIER: US 6248330 B1

** See image for Certificate of Correction **

TITLE: Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides

DATE-ISSUED: June 19, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Labigne; Agnes	Bures sur Yvette			FR
Suerbaum; Sebastien	Bochum			DE
Ferrero; Richard L.	Paris			FR
Thiberge; Jean-Michel	Plaisir			FR

US-CL-CURRENT: 424/192.1; 424/184.1, 424/234.1, 435/6, 435/69.1

CLAIMS:

We claim:

1. An immunogenic composition, which induces antibodies against Helicobacter infection, comprising a purified, synthetic, or recombinant Helicobacter HspA polypeptide or a fragment thereof, wherein said fragment has at least 6 amino acids and is immunogenic.
2. The immunogenic composition according to claim 1, wherein the Hsp polypeptide has the amino acid sequence depicted in FIG. 6 (SEQ ID NO: 29), or a fragment thereof, wherein said fragment has at least 6 amino acids and is immunogenic.
3. The immunogenic composition according to claim 1, wherein the HspA is encoded by the HspA gene of plasmid pILL689 (CNCM I-1356) or a fragment thereof, wherein said fragment has at least 6 amino acids and is immunogenic.
4. The immunogenic composition according to claim 1, further comprising a Helicobacter HspB polypeptide or a fragment thereof, wherein said fragment has at least 6 amino acids and is immunogenic.
5. The immunogenic composition according to claim 4, wherein the HspB is encoded by the HspB gene of plasmid pILL689 (CNCM I-1356) or a fragment thereof, wherein said fragment has at least 6 amino acids and is immunogenic.
6. Proteinaceous material comprising purified, synthetic, or recombinant HspA of Helicobacter pylori or a fragment thereof, wherein said fragment has at

least 6 amino acids and is immunogenic.

7. The proteinaceous material according to claim 6, wherein the material comprises the Helicobacter HspA polypeptide having the amino acid sequence illustrated in FIG. 6 (SEQ ID NO: 29) or a fragment thereof, wherein said fragment has at least 6 amino acids and is immunogenic.

8. The proteinaceous material according to claim 6, wherein the material comprises HspA C-terminal sequence:

GSCCHTGNHDHKHAKHEACCHDHKKH

(SEQ ID NO: 1) or a fragment thereof, wherein said fragment has at least 6 amino acids and is immunogenic.

9. The proteinaceous material according to claim 6 further comprising a Helicobacter HspB polypeptide or a fragment thereof, wherein said fragment has at least 6 amino acids and is immunogenic.

10. Proteinaceous material comprising a fusion protein, wherein the fusion protein comprises at least one Helicobacter HspA or a fragment thereof as defined in any one of claims 6-9 and at least one polypeptide selected from the group consisting of

a Helicobacter pylori urease structural polypeptide or fragment thereof, wherein said fragment is recognized by antibodies to *H. felis* urease, and

a Helicobacter felis urease structural polypeptide or immunogenic fragment thereof.

11. An immunogenic composition, which induces antibodies against Helicobacter infection, comprising at least one sub-unit of a purified, synthetic, or recombinant Helicobacter felis urease structural polypeptide selected from the group of polypeptides consisting of SEQ ID NO: 20 and SEQ ID NO: 21, and a heat shock protein (Hsp) from Helicobacter or a fragment thereof, wherein the Hsp protein is HspA or HspA and HspB encoded by the HspA/HspB genes of plasmid pILL689 (CNCM I-1356), and wherein said fragment has at least 6 amino acids and is immunogenic.

12. The immunogenic composition according to claim 11, wherein the Hsp protein is Helicobacter HspA or Hsp A and HspB having amino acid sequence(s) depicted in FIG. 6 (SEQ ID NOS: 29-30), or a fragment thereof, wherein said fragment has at least 6 amino acids and is immunogenic.

13. The immunogenic composition according to claim 11 or claim 12, which induces protective antibodies.

14. A pharmaceutical composition comprising the immunogenic composition of any one of claims 1-5, 11 or 12, in combination with physiologically acceptable excipient(s) and, optionally, further comprising a pharmaceutically acceptable adjuvant.

15. A method for treatment or prevention of Helicobacter infection in a mammal comprising the step of administering the immunogenic composition of claim 13 to said mammal.

16. An immunogenic composition, capable of inducing antibodies against

- Helicobacter infection, comprising at least one sub-unit of a purified, synthetic, or recombinant Helicobacter felis urease structural polypeptide selected from the group of polypeptides consisting of SEQ ID NO: 20 and SEQ ID NO: 21, further comprising at least one heat shock protein (Hsp) from Helicobacter, wherein the Hsp protein is HspA, HspB, or HspA and HspB encoded by the HspA/HspB genes of plasmid pILL689 (CNCM I-1356), or a fragment thereof, wherein said fragment has at least 6 amino acids and is capable of generating antibodies.

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US 20040052812A1

(19) **United States**(12) **Patent Application Publication** (10) **Pub. No.: US 2004/0052812 A1**
Hoe et al. (43) **Pub. Date: Mar. 18, 2004**(54) **HEAT SHOCK PROTEIN-BASED ANTIVIRAL VACCINES**

(57)

ABSTRACT

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(30) Foreign Application Priority Data

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Publication Classification(51) Int. Cl.⁷ A61K 39/12

(52) U.S. Cl. 424/186.1

The present invention relates to the use of non-pathogenic multi-component viral particles in vaccines which utilize heat shock proteins to enhance the anti-viral immune response. The multi-component viral particles are covalently conjugated to one or more species of "javelin", where javelins are molecules which form non-covalent associations with heat shock proteins. In view of the role of heat shock proteins in the recognition, by the immune system, of antigens, the addition of a javelin "tether" to a multi-component viral particle facilitates complex formation between the particle and a heat shock protein and hence promotes development of an immune reaction to the particle, without requiring the identification of specific epitopes. In addition, the present invention provides for methods of preventing or ameliorating viral infections comprising administering a "javelinized" multi-component viral particle vaccine to a subject at risk of contracting a viral infection or who has already been infected. Because of the diversity of epitopes in the multi-component viral particles, a single vaccine formulation may be used to promote immunity toward multiple viral strains in subjects having various histocompatibility profiles.

DOCUMENT-IDENTIFIER: US 20040052812 A1

TITLE: Heat shock protein-based antiviral vaccines

Abstract Paragraph:

The present invention relates to the use of non-pathogenic multi-component viral particles in vaccines which utilize heat shock proteins to enhance the anti-viral immune response. The multi-component viral particles are covalently conjugated to one or more species of "javelin", where javelins are molecules which form non-covalent associations with heat shock proteins. In view of the role of heat shock proteins in the recognition, by the immune system, of antigens, the addition of a javelin "tether" to a multi-component viral particle facilitates complex formation between the particle and a heat shock protein and hence promotes development of an immune reaction to the particle, without requiring the identification of specific epitopes. In addition, the present invention provides for methods of preventing or ameliorating viral infections comprising administering a "javelinized" multi-component viral particle vaccine to a subject at risk of contracting a viral infection or who has already been infected. Because of the diversity of epitopes in the multi-component viral particles, a single vaccine formulation may be used to promote immunity toward multiple viral strains in subjects having various histocompatibility profiles

DOCUMENT-IDENTIFIER: US 20040047879 A1

TITLE: Hepatitis b virus (hbv) antigenic polypeptide-heat shock protein complex and use thereof

Abstract Paragraph:

The present invention provides a kind of HBV-antigen bound to heat shock proteins (hsps) which comprises core antigen, surface antigen and polymerase antigen. The present invention also provides a complex of HBV antigen bound to heat shock protein gp96 and hsp78, as well as a method for preparing the complex. The complex includes a complex of gp96 and hsp78 non-covalently bound to antigenic polypeptide, as well as a fusion protein of both which results from covalent binding. Such complex can be used to prepare therapeutic vaccine for treating hepatitis B and primary hepatocellular carcinoma.

CLAIMS:

7. A use of the complex of claim 1 in preparing a therapeutic vaccine for treating hepatitis B and primary hepatocellular carcinoma, wherein the complex comprises the HBV antigen and the heat shock protein gp96 or hsp78.

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US 20040022796A1

(19) **United States**(12) **Patent Application Publication** (10) **Pub. No.: US 2004/0022796 A1**
Srivastava (43) **Pub. Date: Feb. 5, 2004**(54) **USING HEAT SHOCK PROTEINS AND
ALPHA-2-MACROGLOBULINS TO
INCREASE THE IMMUNE RESPONSE TO
VACCINES COMPRISING HEAT SHOCK
PROTEIN-PEPTIDE COMPLEXES OR
ALPHA-2-MACROGLOBULIN-PEPTIDE
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2, 2002.****Publication Classification**(51) **Int. Cl.⁷ A61K 39/00**(52) **U.S. Cl. 424/185.1**(57) **ABSTRACT**

The present invention provides a method of improving or prolonging a subject's immune response to a vaccine composition comprising heat shock protein (HSP)-peptide complexes or alpha-2-macroglobulin (α 2M)-peptide complexes (hereinafter "HSP/ α 2M vaccine composition"). The HSP-peptide complexes or α 2M-peptide complexes of the vaccine composition comprise HSP(s) or α 2M complexed to a component against which an immune response is desired to be induced. In particular the invention is directed to methods of improving or prolonging a subject's immune response comprising administering an HSP/ α 2M vaccine composition in conjunction with a preparation comprising HSP or α 2M, alone or complexed to a peptide that is not the component against which an immune response is desired to be induced (hereinafter "HSP/ α 2M preparation"), i.e., the HSP/ α 2M preparation does not display the immunogenicity of the component. In particular, HSP/ α 2M vaccine compositions are administered in conjunction with HSP/ α 2M preparation to improve or prolong the immune response of a subject against an infectious disease or cancer.

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File: PGPB

Feb 5, 2004

PGPUB-DOCUMENT-NUMBER: 20040022796

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040022796 A1

TITLE: Using heat shock proteins and alpha-2-macroglobulins to increase the immune response to vaccines comprising heat shock protein-peptide complexes or alpha-2-macroglobulin-peptide complexes

PUBLICATION-DATE: February 5, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Srivastava, Pramod K.	Avon	CT	US

US-CL-CURRENT: 424/185.1

CLAIMS:

What is claimed is:

1. A method for inducing an immune response in a subject comprising the steps of: (a) administering to the subject an HSP/.alpha.2M vaccine composition comprising an HSP or .alpha.2M complexed to a component against which an immune response is desired to be induced; and (b) administering to the subject a heat shock protein preparation, wherein the heat shock protein preparation does not display the immunogenicity of the component, and wherein the heat shock protein preparation and HSP/.alpha.2M vaccine composition are not present in admixture; such that an immune response to the component is produced in the subject.

2. A method of inducing an immune response by an HSP/.alpha.2M vaccine composition in a subject comprising the steps of: (a) administering to the subject a heat shock protein preparation; and (b) administering to the subject an HSP/.alpha.2M vaccine composition comprising an HSP or .alpha.2M complexed to a component against which an immune response is desired to be induced, the HSP/.alpha.2M vaccine composition being in an amount that is sub-immunogenic for the component in the absence of step (a), such that an immune response to the component is induced in the subject, and wherein the heat shock protein preparation does not display the immunogenicity of the component.

3. The method of claim 2, wherein the HSP/.alpha.2M vaccine composition and the heat shock protein preparation are not present in admixture.

4. A method of treating or preventing an infectious disease in a subject comprising the steps of: (a) administering to the subject an HSP/.alpha.2M vaccine composition comprising an HSP or .alpha.2M complexed to a component that displays the antigenicity of an antigen of an infectious agent that causes the infectious disease; and (b) administering to the subject an amount of a heat shock protein preparation effective in combination with step (a) to induce or increase an immune

response to the component in the subject, wherein the heat shock protein preparation does not display the immunogenicity of the component, and wherein the HSP preparation and HSP/.alpha.2M vaccine composition are not present in admixture.

5. A method of treating or preventing a cancer in a subject comprising the steps of: (a) administering to the subject an HSP/.alpha.2M vaccine composition comprising an HSP or .alpha.2M complexed to a component that displays the antigenicity of a tumor specific or tumor associated antigen of a cancer cell; and (b) administering to the subject an amount of a heat shock protein preparation effective to induce or increase an immune response in the subject to the component, wherein the heat shock protein preparation does not display the immunogenicity of the component, and wherein the HSP preparation and HSP/.alpha.2M vaccine composition are not present in admixture.

6. The method of claim 1, wherein the immune response to the component produced in the subject is increased relative to the immune response to the component in the subject in the absence of step (b).

7. The method of claim 1 wherein the heat shock protein preparation comprises a heat shock protein selected from the group consisting of hsp70, hsp90, gp96, hsp110, grp170, calreticulin, and a combination thereof.

8. The method of claim 2 wherein the heat shock protein preparation comprises a heat shock protein selected group consisting of hsp70, hsp90, gp96, hsp110, grp170, calreticulin, and a combination thereof.

9. The method of claim 4 wherein the heat shock protein preparation comprises a heat shock protein selected from the group consisting of hsp70, hsp90, gp96, hsp110, grp170, calreticulin, and a combination thereof.

10. The method of claim 5 wherein the heat shock protein preparation comprises a heat shock protein selected from the group consisting of hsp70, hsp90, gp96, hsp110, grp170, calreticulin, and a combination thereof.

11. The method of claim 1 wherein the heat shock protein preparation comprises heat shock protein-peptide complexes.

12. The method of claim 2 wherein the heat shock protein preparation comprises heat shock protein-peptide complexes.

13. The method of claim 4 wherein the heat shock protein preparation comprises heat shock protein-peptide complexes.

14. The method of claim 5 wherein the heat shock protein preparation comprises heat shock protein-peptide complexes.

15. The method of claim 1 wherein the heat shock protein preparation comprises purified heat shock proteins.

16. The method of claim 2 wherein the heat shock protein preparation comprises purified heat shock proteins.

17. The method of claim 4 wherein the heat shock protein preparation comprises purified heat shock proteins.

18. The method of claim 5 wherein the heat shock protein preparation comprises

purified heat shock proteins.

19. The method of claim 1 wherein the subject is human and the heat shock protein preparation comprises mammalian heat shock proteins.

20. The method of claim 2 wherein the subject is human and the heat shock protein preparation comprises mammalian heat shock proteins.

21. The method of claim 4 wherein the subject is human and the heat shock protein preparation comprises mammalian heat shock proteins.

22. The method of claim 5 wherein the subject is human and the heat shock protein preparation comprises mammalian heat shock proteins.

23. The method of claim 1, 2, 4, or 5 wherein the heat shock protein preparation is administered before the administration of the HSP/.alpha.2M vaccine composition.

24. The method of claim 1, 2, 4, or 5 wherein the heat shock protein preparation is administered concurrently with the administration of the HSP/.alpha.2M vaccine composition, and the heat shock protein preparation and the HSP/.alpha.2M vaccine composition are not present in admixture.

25. The method of claim 1, 2, 4, or 5 wherein the heat shock protein preparation is administered after the administration of the HSP/.alpha.2M vaccine composition.

26. The method of claim 7, 8, 9, or 10 wherein the heat shock protein preparation is administered before the administration of the HSP/.alpha.2M vaccine composition.

27. The method of claim 7, 8, 9, or 10 wherein the heat shock protein preparation is administered concurrently with the administration of the HSP/.alpha.2M vaccine composition, and the heat shock protein preparation and the HSP/.alpha.2M vaccine composition are not administered in admixture.

28. The method of claim 7, 8, 9, or 10 wherein the heat shock protein preparation is administered after the administration of the HSP/.alpha.2M vaccine composition.

29. The method of claim 11, 12, 13 or 14 wherein the heat shock protein preparation is administered before the administration of the HSP/.alpha.2M vaccine composition.

30. The method of claim 11, 12, 13 or 14 wherein the heat shock protein preparation is administered concurrently with the administration of the HSP/.alpha.2M vaccine composition, and the heat shock protein preparation and the HSP/.alpha.2M vaccine composition are not administered in admixture.

31. The method of claim 11, 12, 13 or 14 wherein the heat shock protein preparation is administered after the administration of the HSP/.alpha.2M vaccine composition.

32. The method of claim 15, 16, 17 or 18 wherein the heat shock protein preparation is administered before the administration of the HSP/.alpha.2M vaccine composition.

33. The method of claim 15, 16, 17 or 18 wherein the heat shock protein preparation is administered concurrently with the administration of the HSP/.alpha.2M vaccine composition, and the heat shock protein preparation and the HSP/.alpha.2M vaccine composition are not administered in admixture.

34. The method of claim 15, 16, 17 or 18 wherein the heat shock protein preparation

is administered after the administration of the HSP/.alpha.2M vaccine composition.

35. The method of claim 4 wherein the infectious disease is selected from the group consisting of hepatitis A virus, hepatitis B virus, hepatitis C virus, influenza, varicella, adenovirus, herpes simplex I virus, herpes simplex II virus, rinderpest, rhinovirus, ECHO virus, rotavirus, respiratory syncytial virus, papilloma virus, papova virus, cytomegalovirus, echinovirus, arbovirus, hantavirus, coxsackie virus, mumps virus, measles virus, rubella virus, polio virus, human immunodeficiency virus type I (HIV-I), human immunodeficiency virus type II (HIV-II), mycobacteria, rickettsia, mycoplasma, neisseria, legionella, leishmania, kokzidioa, trypanosoma and chlamydia.

36. The method of claim 5 wherein the cancer is selected from the group consisting of fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma, leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, myeloblastic leukemia, promyelocytic leukemia, myelomonocytic leukemia, monocytic leukemia and erythroleukemia, chronic leukemia, chronic myelocytic leukemia, chronic granulocytic leukemia, chronic lymphocytic leukemia, polycythemia vera, lymphoma, Hodgkin's disease lymphoma, non-Hodgkin's disease lymphoma, multiple myeloma, Waldenstrom's macroglobulinemia, and heavy chain disease.

37. The method of claim 4, wherein the method is for preventing an infectious disease.

38. The method of claim 5, wherein the method is for treating a cancer.

39. The method of claim 5, wherein the method is for preventing a cancer.

40. A kit comprising: (a) a first container containing a heat shock protein preparation or an .alpha.2M preparation, in an amount effective to increase an immune response elicited by an HSP/.alpha.2M vaccine composition against a component of the HSP/.alpha.2M vaccine composition against which an immune response is desired; and (b) a second container containing the HSP/.alpha.2M vaccine composition in an amount that, when administered before, concurrently with, or after the administration of the heat shock protein preparation of (a), is effective to induce an immune response against the component.

41. The kit of claim 40 wherein the first container contains a heat shock protein preparation comprising a heat shock protein selected from the group consisting of hsp70, hsp90, gp96, hsp110, grp170, calreticulin, and a combination thereof.

42. The kit of claim 40 wherein the first container contains a heat shock protein preparation comprising heat shock protein-peptide complexes.

43. The kit of claim 40 wherein the first container contains a heat shock protein preparation comprising purified heat shock proteins.

44. The kit of claim 40 wherein the first container contains a heat shock protein preparation comprising heat shock protein-peptide complexes and purified heat shock proteins.

45. The kit of claim 40 wherein the first container contains a heat shock protein preparation comprising mammalian heat shock proteins.

46. The kit of claim 40 wherein the amount of HSP/.alpha.2M vaccine composition in the second container is insufficient for inducing an immune response in a subject in the absence of administering the heat shock protein preparation or .alpha.2M preparation in the first container.

47. A method for inducing an immune response in a subject comprising the steps of: (a) administering to the subject an HSP/.alpha.2M vaccine composition comprising an HSP or .alpha.2M complexed to a component against which an immune response is desired to be induced; and (b) administering to the subject an .alpha.2M preparation, wherein the .alpha.2M preparation does not display the immunogenicity of the component, and wherein the .alpha.2M preparation and the HSP/.alpha.2M vaccine composition are not present in admixture; such that an immune response to the component is produced in the subject.

48. A method of inducing an immune response by an HSP/.alpha.2M vaccine composition in a subject comprising the steps of: (a) administering to the subject an .alpha.2M preparation; and (b) administering to the subject an HSP/.alpha.2M vaccine composition comprising an HSP or .alpha.2M complexed to a component against which an immune response is desired to be induced, the HSP/.alpha.2M vaccine composition being in an amount that is sub-immunogenic for the component in the absence of step (a), such that an immune response to the component is induced in the subject, and wherein the .alpha.2M preparation does not display the immunogenicity of the component.

49. A method of treating or preventing an infectious disease in a subject comprising the steps of: (a) administering to the subject an HSP/.alpha.2M vaccine composition comprising an HSP or .alpha.2M complexed to a component that displays the antigenicity an antigen of an infectious agent that causes the infectious disease; and (b) administering to the subject an amount of an .alpha.2M preparation effective in combination with step (a) to induce or increase an immune response to the component in the subject, wherein the .alpha.2M preparation does not display the immunogenicity of the component, and wherein the HSP/.alpha.2M vaccine composition and .alpha.2M preparation are not present in admixture.

50. A method of treating or preventing a cancer in a subject comprising the steps of: (a) administering to the subject an HSP/.alpha.2M vaccine composition comprising an HSP or .alpha.2M complexed to a component that displays the antigenicity of a tumor specific or tumor associated antigen of a cancer cell; and (b) administering to the subject an amount of an .alpha.2M preparation effective to induce or increase an immune response in the subject to the component, wherein the .alpha.2M preparation does not display the immunogenicity of the component, and wherein the HSP/.alpha.2M vaccine composition and .alpha.2M preparation are not present in admixture.

51. The method of claim 47, wherein the immune response to the component produced in the subject is increased relative to the immune response to the component in the subject in the absence of step (b).

52. The method of claim 48, wherein the HSP/.alpha.2M vaccine composition and the .alpha.2M preparation are not present in admixture.

- 53. The method of claim 47, 48, 49, or 50 wherein the .alpha.2M preparation comprises .alpha.2M-peptide complexes.
- 54. The method of claim 47, 48, 49, or 50 wherein the .alpha.2M preparation comprises purified .alpha.2M.
- 55. The method of claim 47, 48, 49, or 50 wherein the subject is human and the .alpha.2M preparation comprises mammalian .alpha.2M.

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US 20030211102A1

(19) **United States**
(12) **Patent Application Publication** (10) **Pub. No.: US 2003/0211102 A1**
Tiwari (43) **Pub. Date: Nov. 13, 2003**

(54) **IMMUNOGENS FOR TREATMENT OF
NEOPLASTIC AND INFECTIOUS DISEASE**

Publication Classification

(76) **Inventor: Raj Tiwari, Bellerose, NY (US)**

(51) **Int. Cl.⁷** **C12Q 1/70; A61K 39/395;
C07K 16/18; C12P 21/02;
C12N 5/06**
(52) **U.S. Cl.** **424/145.1; 435/69.1; 435/320.1;
435/5; 530/388.25; 435/337**

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(21) **Appl. No.: 10/379,462**

(22) **Filed: Mar. 3, 2003**

Related U.S. Application Data

(60) **Provisional application No. 60/360,720, filed on Mar.
1, 2002.**

(57) **ABSTRACT**

The present invention relates to prophylactic and therapeutic methods of immunization against neoplastic and infectious diseases. The invention provides a method for identification of novel immunogens and compositions of such immunogens that are useful for eliciting immune responses against antigens associated with neoplastic or infectious diseases.

DOCUMENT-IDENTIFIER: US 20030211102 A1

TITLE: Immunogens for treatment of neoplastic and infectious disease

CLAIMS:

26. A method of making a vaccine against a target cell or an antigen thereof, comprising: a) isolating first binding substances that specifically bind to heat shock protein-peptide complexes differentially expressed by the target cell relative to a second cell; b) isolating mimotopes that specifically bind to the first binding substances; and c) incorporating the mimotopes into the vaccine.



US 2003017665A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2003/0176665 A1**

Scholz et al.

(43) **Pub. Date: Sep. 18, 2003**

(54) **SOLUBLE COMPLEXES OF TARGET
PROTEINS AND PEPTIDYL PROLYL
ISOMERASE CHAPERONES AND METHODS
OF MAKING AND USING THEM**

Aug. 31, 2001 (EP) 01120939.2

Publication Classification

(51) **Int. Cl.⁷** **C07K 14/15; C12P 21/06**

(52) **U.S. Cl.** **530/395; 435/68.1**

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(21) **Appl. No.: 10/179,905**

(22) **Filed: Jun. 24, 2002**

(30) **Foreign Application Priority Data**

Jun. 22, 2001 (EP) 01115225.3

(57) **ABSTRACT**

The present invention relates to the diagnosis of HIV infections. It especially teaches the production of a soluble retroviral surface glycoprotein- (or transmembrane glycoprotein)-chaperone complex and the advantageous use of a chaperone-antigen complex especially in the detection of antibodies to HIV in immunoassays, preferably according to the double antigen bridge concept, or as an immunogen. The invention also discloses soluble complexes comprising a variant of HIV-1 gp41 or a variant of HIV-2 gp36, respectively, and a chaperone selected from the peptidyl-prolyl-isomerase class of chaperones. Variants comprising specific amino-acid substitutions in the N-helical domain of HIV-1 gp41 or of HIV-2 gp36, respectively, are also described.

DOCUMENT-IDENTIFIER: US 20030176665 A1

TITLE: Soluble complexes of target proteins and peptidyl prolyl isomerase chaperones and methods of making and using them

CLAIMS:

40. A method of eliciting an immune response, comprising: injecting into a subject a vaccine comprising a soluble retroviral surface glycoprotein-chaperone complex thereby eliciting antibodies in a subject that bind the retroviral surface glycoprotein.



US 20030165516A1

(19) **United States**

(12) **Patent Application Publication**
Srivastava

(10) **Pub. No.: US 2003/0165516 A1**

(43) **Pub. Date: Sep. 4, 2003**

(54) **STRESS PROTEIN-PEPTIDE COMPLEXES
AS PROPHYLACTIC AND THERAPEUTIC
VACCINES AGAINST INTRACELLULAR
PATHOGENS**

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(21) **Appl. No.: 10/180,593**

(22) **Filed: Jun. 25, 2002**

Related U.S. Application Data

(60) **Division of application No. 09/412,420, filed on Oct.
5, 1999, now Pat. No. 6,455,503, which is a continu-**

ation of application No. 08/704,727, filed on Jun. 19,
1997, now Pat. No. 6,048,530, filed as 371 of inter-
national application No. PCT/US95/03311, filed on
Mar. 16, 1995, which is a continuation-in-part of
application No. 08/210,421, filed on Mar. 16, 1994,
now Pat. No. 5,961,979.

Publication Classification

(51) **Int. Cl.⁷ A61K 39/00**

(52) **U.S. Cl. 424/185.1**

(57) **ABSTRACT**

Disclosed is a family of vaccines that contain stress protein-peptide complexes which when administered to a mammal are operative to initiate in the mammal a cytotoxic T cell response against cells infected with a preselected intracellular pathogen. Also disclosed are methodologies for preparing and administering vaccines containing such stress protein-peptide complexes.

DOCUMENT-IDENTIFIER: US 20030165516 A1

TITLE: Stress protein-peptide complexes as prophylactic and therapeutic vaccines against intracellular pathogens

Abstract Paragraph:

Disclosed is a family of vaccines that contain stress protein-peptide complexes which when administered to a mammal are operative to initiate in the mammal a cytotoxic T cell response against cells infected with a preselected intracellular pathogen. Also disclosed are methodologies for preparing and administering vaccines containing such stress protein-peptide complexes.

CLAIMS:

1. A vaccine for administration to a mammal for inducing in the mammal a cytotoxic T cell response against a preselected intracellular pathogen, the vaccine comprising: (a) an immunogenic stress protein-peptide complex operative to initiate in said mammal a cytotoxic T cell response against said pathogen, said complex comprising, a peptide that is present in a eukaryotic cell infected with said pathogen but not present in said cell when said cell is not infected with said pathogen, complexed with a stress protein; and (b) a pharmaceutically acceptable carrier.

2. A vaccine for administration to a mammal for inducing in said mammal resistance to infection by a preselected intracellular pathogen, the vaccine comprising: (a) an immunogenic stress protein-peptide complex operative to initiate in said mammal, by means of a cytotoxic T cell response in said mammal, resistance to infection by said pathogen, said complex comprising, a peptide that is present in a eukaryotic cell infected with said pathogen but not present in said cell when said cell is not infected with said pathogen, complexed with a stress protein; and (b) a pharmaceutically acceptable carrier.

18. A method of inducing in a mammal a cytotoxic T cell response against a preselected intracellular pathogen that causes disease in said mammal, the method comprising: administering to said mammal a vaccine comprising, (a) an immunogenic stress protein-peptide complex operative to initiate in said mammal a cytotoxic T cell response against said pathogen and comprising, a peptide that is present in a eukaryotic cell infected with said pathogen but not present in said cell when said cell is not infected with said pathogen, complexed with a stress protein, and (b) a pharmaceutically acceptable carrier, in an amount sufficient to elicit in said mammal a cytotoxic T cell response against said pathogen.

19. A method of inducing in a mammal resistance to infection by a preselected intracellular pathogen that-causes disease in said mammal, the method comprising: administering to said mammal a vaccine comprising, (a) an immunogenic stress protein-peptide complex operative to initiate in said mammal cytotoxic T cell response against said pathogen and comprising, a peptide that is present in a eukaryotic cell infected with said pathogen but not present in said cell when said cell is not infected with said pathogen, complexed with a stress protein, and (b) a pharmaceutically acceptable carrier, in an amount sufficient to induce in said mammal, by means of the cytotoxic T cell response in said mammal, resistance to infection by said pathogen.

31. A method for preparing a vaccine for inducing in a mammal a cytotoxic T cell response against a preselected intracellular pathogen, the method comprising: (a) harvesting from a eukaryotic cell infected with said pathogen an immunogenic stress protein-peptide complex comprising, a peptide that is present in said cell infected with said pathogen but not present in said cell when said cell is not infected with said pathogen complexed with a stress protein, said complex, when administered to said mammal, being operative at initiating in said mammal a cytotoxic T cell response against said pathogen; and (b) combining said complex with a pharmaceutically acceptable carrier.

32. A method for preparing a vaccine for inducing in a mammal a cytotoxic T cell response against a preselected intracellular pathogen, the method comprising: (a) reconstituting in vitro, a peptide that is present in a eukaryotic cell infected with said pathogen but not present in said cell when said cell is not infected with said pathogen and a stress protein, thereby to generate a stress protein-peptide complex, which when administered to said mammal is operative to initiate a cytotoxic T cell response against said pathogen in said mammal; and (b) combining said complex with a pharmaceutically acceptable carrier.

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US006048530A

United States Patent [19]
Srivastava

[11] **Patent Number:** **6,048,530**
 [45] **Date of Patent:** ***Apr. 11, 2000**

- [54] **STRESS PROTEIN-PEPTIDE COMPLEXES AS PROPHYLACTIC AND THERAPEUTIC VACCINES AGAINST INTRACELLULAR PATHOGENS**
- [75] **Inventor:** **Pramod K. Srivastava, Riverdale, N.Y.**
- [73] **Assignee:** **Mount Sinai School of Medicine of New York University, New York, N.Y.**
- [*] **Notice:** This patent is subject to a terminal disclaimer.
- [21] **Appl. No.:** **08/704,727**
- [22] **PCT Filed:** **Mar. 16, 1995**
- [86] **PCT No.:** **PCT/US95/03311**
§ 371 Date: **Jun. 19, 1997**
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Related U.S. Application Data

- [63] **Continuation-in-part of application No. 08/210,421, Mar. 16, 1994, Pat. No. 5,961,979.**
- [51] **Int. Cl.⁷** **A61K 39/395; A61K 39/385; A61K 39/12; A61K 39/02**
- [52] **U.S. Cl.** **424/193.1; 424/194.1; 424/196.11; 424/197.11; 424/278.1; 424/281.1; 424/282.2; 424/265.1; 424/274.1; 424/204.1; 424/234.1; 514/21; 530/412; 530/413; 435/69.1**
- [58] **Field of Search** **530/278.1, 412, 530/413; 514/21; 424/281.1, 282.1, 193.1, 194.1, 196.11, 197.11, 265.1, 274.1, 204.1, 234.1; 435/69.1**

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[57] ABSTRACT

Disclosed is a family of vaccines that contain stress protein-peptide complexes which when administered to a mammal are operative to initiate in the mammal a cytotoxic T cell response against cells infected with a preselected intracellular pathogen. Also disclosed are methodologies for preparing and administering vaccines containing such stress protein-peptide complexes.

24 Claims, 3 Drawing Sheets



US006455503B1

(12) **United States Patent**
Srivastava

(10) Patent No.: **US 6,455,503 B1**
(45) Date of Patent: **Sep. 24, 2002**

(54) **STRESS PROTEIN-PEPTIDE COMPLEXES
AS PROPHYLACTIC AND THERAPEUTIC
VACCINES AGAINST INTRACELLULAR
PATHOGENS**

(75) Inventor: **Pramod K. Srivastava, Riverdale, NY
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(22) Filed: **Oct. 5, 1999**

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6,048,530, which is a continuation-in-part of application No.
08/210,421, filed on Mar. 16, 1994, now Pat. No. 5,961,979.

(51) Int. Cl.⁷ **A61K 38/00; C07K 1/00;
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530/344; 530/806; 530/403; 530/300; 530/350;
514/2; 514/12; 436/823**

(58) Field of Search **424/193.1; 530/300,
530/350, 344, 806, 403; 514/2, 12, 21;
436/823**

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(57) ABSTRACT

Disclosed is a family of vaccines that contain stress protein-peptide complexes which when administered to a mammal are operative to initiate in the mammal a cytotoxic T cell response against cells infected with a preselected intracellular pathogen. Also disclosed are methodologies for preparing and administering vaccines containing such stress protein-peptide complexes.

20 Claims, 3 Drawing Sheets

DGCUMENT-IDENTIFIER: US 6455503 B1

✓ TITLE: Stress protein-peptide complexes as prophylactic and therapeutic vaccines against intracellular pathogens

Abstract Text (1):

Disclosed is a family of vaccines that contain stress protein-peptide complexes which when administered to a mammal are operative to initiate in the mammal a cytotoxic T cell response against cells infected with a preselected intracellular pathogen. Also disclosed are methodologies for preparing and administering vaccines containing such stress protein-peptide complexes.

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(19) **United States**

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Langermann et al. (43) Pub. Date: **May 29, 2003**

(54) **CHAPERONE AND ADHESIN PROTEINS;
VACCINES, DIAGNOSTICS AND METHOD
FOR TREATING INFECTIONS**

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(21) Appl. No.: **10/288,978**

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(60) Provisional application No. 60/082,824, filed on Apr.
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Publication Classification

(51) Int. Cl.⁷ **A61K 39/40; A61K 39/02**
(52) U.S. Cl. **424/190.1; 424/164.1**

(57) **ABSTRACT**

The present invention provides bacterial immunogenic agents for administration to humans and non-human animals to stimulate an immune response. It particularly relates to the vaccination of mammalian species with heteropolymeric protein complexes as a mechanism for stimulating production of antibodies that protect the vaccine recipient against infection by pathogenic bacterial species. In another aspect the invention provides antibodies against such proteins and protein complexes that may be used as diagnostics and/or as protective/treatment agents for pathogenic bacterial species. A novel vector for expressing the FimC-H complex at optimal levels is also disclosed.

DOCUMENT-IDENTIFIER: US 20030099665 A1

TITLE: Chaperone and adhesin proteins; vaccines, diagnostics and method for treating infections

CLAIMS:

1. A vaccine against bacterial infections comprising a complex of a bacterial chaperone protein with either an adhesin protein or an immunogenic fragment of said adhesin protein.

17. A method for preventing and/or treating UTIs in a host comprising immunizing said host with a member selected from the group consisting of: (a) a vaccine according to claim 1, and (b) at least one antibody raised against a complex of a bacterial chaperone protein with either an adhesin protein or an immunogenic mannose-binding fragment of said adhesin protein.

adhesin

↓
Fim H/FimC